

# Journal of the Arab Society for Medical Research



# Journal of the Arab Society for Medical Research

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# Journal of the Arab Society for Medical Research

# **Author Guidelines**

#### About the Journal

Journal of The Arab Society for Medical Research (JASMR), is a Semiannual (June and December), Peer-Reviewed, open access, published under the auspices of the Arab Society for Medical Research, at the National Research Centre of Egypt. JASMR is aiming for development the medical research globally in the Arab countries and faster communication between Arab scientists and with the scientists of advanced countries in the field of medical research.

#### Abstracting and Indexing Information

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DOAJ, Index Medicus for the Eastern Mediterranean Region(IMEMR)

#### Scope of the journal

Journal of The Arab Society for Medical Research (JASMR) is online journal; cover all medical field of research such as: Internal Medicine, Clinical Pathology, Biochemistry, Biological Anthropology, Child Health, Pathology, General Surgery, Obstetrics and Gynecology, Dentistry, Pharmacology, Natural products, etc... Paper may be submitted from any discipline of basic, applied, clinical and biological research.

#### The Editorial Process

A manuscript will be reviewed for possible publication with the understanding that it is being submitted to Journal of The Arab Society for Medical Research alone at that point in time and has not been published anywhere, simultaneously submitted, or already accepted for publication elsewhere. The journal expects that authors would authorize one of them to correspond with the Journal for all matters related to the manuscript. All manuscripts received are duly acknowledged. On submission, editors review all submitted manuscripts initially for suitability for formal review and check for plagiarism similarity. Manuscripts with insufficient originality, high similarity index, serious scientific or technical flaws, or lack of a significant message are rejected before proceeding for formal peer-review. Manuscripts that are unlikely to be of interest to the Journal of The Arab Society for Medical Research readers are also liable to be rejected at this stage itself.

Manuscripts after screened by the Editor in Chief and found that are suitable for publication in Journal of The Arab Society for Medical Research are sent to two or more expert reviewers. The reviewers selected criteria are full professor degree and who are the oldest professor with high expertise and consultant in their field (sometimes selected the oldest associate professor in some rare fields of specialty). Moreover, at least one reviewer must be a full professor out of NRC, Egypt. The journal follows a double-blind review process, wherein the reviewers and authors are unaware of each other's identity. All reviewers and/or the editorial board members who are authors and/or have any conflict of interest will be excluded from revision and publication decisions. During submission, the contributor is requested to provide names of two or three qualified reviewers who have experience in the subject of the submitted manuscript, but this is not mandatory. However, the selection of these reviewers is at the sole discretion of the Editor-in-Chief.

The comments and suggestions (acceptance/ rejection/ amendments in manuscript) received from reviewers are conveyed to the corresponding author. If required, the author is requested to provide a point by point response to reviewers' comments and submit a revised version of the manuscript. This process is repeated till reviewers and editors are satisfied with the manuscript.

Manuscripts accepted for publication are copy edited for grammar, punctuation, print style, and format. Page proofs are sent to the corresponding author. The corresponding author is expected to return the corrected proofs within three days. It may not be possible to incorporate corrections received after that period. The whole process of submission of the manuscript to final decision and sending and receiving proofs is completed online. To achieve faster and greater dissemination of knowledge and information, the journal publishes articles online as 'Ahead of Print' immediately on acceptance.

#### **Clinical trial registry**

Journal of The Arab Society for Medical Research favors registration of clinical trials and is a signatory to the Statement on publishing clinical trials in Indian biomedical journals. Journal of The Arab Society for Medical Research would publish clinical trials that have been registered with a clinical trial registry that allows free online access to public. Registration in the following trial registers is acceptable: http://www.ctri.in/; http://www.actr.org.au/; http://www.clinicaltrials.gov/; http:// isrctn.org/; http://www.trialregister.nl/trialreg/index.asp; and http://www.umin.ac.jp/ctr. This is applicable to clinical trials that have begun enrollment of subjects in or after June 2008. Clinical trials that have commenced enrollment of subjects prior to June 2008 would be considered for publication in Journal of The Arab Society for Medical Research only if they have been registered retrospectively with clinical trial registry that allows unhindered online access to public without charging any fees.

#### Authorship Criteria

Authorship credit should be based only on substantial contributions to each of the three components mentioned below:

- 1. Concept and design of study or acquisition of data or analysis and interpretation of data;
- 2. Drafting the article or revising it critically for important intellectual content; and
- 3. Final approval of the version to be published.

Participation solely in the acquisition of funding or the collection of data does not justify authorship. General supervision of the research group is not sufficient for authorship. Each contributor should have participated sufficiently in the work to take public responsibility for appropriate portions of the content of the manuscript. The order of naming the contributors should be based on the relative contribution of the contributor towards the study and writing the manuscript. Once submitted the order cannot be changed without written consent of all the contributors. The journal prescribes a maximum number of authors for manuscripts depending upon the type of manuscript, its scope and number of institutions involved (vide infra). The authors should provide a justification, if the number of authors exceeds these limits.

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6. Registration number in case of a clinical trial and where it is registered (name of the registry and its URL)

7. Conflicts of Interest of each author/ contributor. A statement of financial or other relationships that might lead to a conflict of interest, if that information is not included in the manuscript itself or in an authors' form

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#### **Preparation of Manuscripts**

Manuscripts must be prepared in accordance with "Uniform requirements for Manuscripts submitted to Biomedical Journals" developed by the International Committee of Medical Journal Editors (October 2008). The uniform requirements and specific requirement of Journal of The Arab Society for Medical Research are summarized below. Before submitting a manuscript, contributors are requested to check for the latest instructions available. Instructions are also available from the website of the journal (http://www.asmr.eg.net) and from the manuscript submission site http://www.journalonweb.com/jasmr).

Journal of The Arab Society for Medical Research accepts manuscripts written in American English.

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#### **Types of Manuscripts**

#### **Original articles:**

These include randomized controlled trials, intervention studies, studies of screening and diagnostic test, outcome studies, cost effectiveness analyses, case-control series, and surveys with high response rate. The text of original articles amounting to up to 3000 words (excluding Abstract, references and Tables) should be divided into sections with the headings Abstract, Key-words, Introduction, Material and Methods, Results, Discussion, References, Tables and Figure legends.

Introduction: State the purpose and summarize the rationale for the study or observation.

#### Materials and Methods: It should include and describe the following aspects:

Ethics: When reporting studies on human beings, indicate whether the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the Helsinki Declaration of 1975, as revised in 2000 (available at http://www.wma.net/e/policy/17-c\_e.html). For prospective studies involving human participants, authors are expected to mention about approval of (regional/ national/ institutional or independent Ethics Committee or Review Board, obtaining informed consent from adult research participants and obtaining assent for children aged over 7 years participating in the trial. The age beyond which assent would be required could vary as per regional and/ or national guidelines. Ensure confidentiality of subjects by desisting from mentioning participants' names, initials or hospital numbers, especially in illustrative material. When reporting experiments on animals, indicate whether the institution's or a national research council's guide for, or any national law on the care and use of laboratory animals was followed. Evidence for approval by a local Ethics Committee (for both human as well as animal studies) must be supplied by the authors on demand. Animal experimental procedures should be as humane as possible and the details of anesthetics and analgesics used should be clearly stated. The ethical standards of experiments must be in accordance with the guidelines provided by the CPCSEA and World Medical Association Declaration of Helsinki on Ethical Principles for Medical Research Involving Humans for studies involving experimental animals and human beings, respectively). The journal will not consider any paper which is ethically unacceptable. A statement on ethics committee permission and ethical practices must be included in all research articles under the 'Materials and Methods' section.

#### Study design:

Selection and Description of Participants: Describe your selection of the observational or experimental participants (patients or laboratory animals, including controls) clearly, including eligibility and exclusion criteria and a description of the source population. *Technical information:* Identify the methods, apparatus (give the manufacturer's name and address in parentheses), and procedures in sufficient detail to allow other workers to reproduce the results. Give references to established methods, including statistical methods (see below); provide references and brief descriptions for methods that have been published but are not well known; describe new or substantially modified methods, give reasons for using them, and evaluate their limitations. Identify precisely all drugs and chemicals used, including generic name(s), dose(s), and route(s) of administration.

Reports of randomized clinical trials should present information on all major study elements, including the protocol, assignment of interventions (methods of randomization, concealment of allocation to treatment groups), and the method of masking (blinding), based on the CONSORT Statement (http://www.consort-statement.org).

#### **Reporting Guidelines for Specific Study Designs**

Initiative	Type of Study	Source
CONSORT	Randomized controlled trials	http://www.consort-statement.org
STARD	Studies of diagnostic accuracy	http://www.consort-statement.org/stardstatement.htm
QUOROM	Systematic reviews and meta-analyses	http://www.consort- statement.org/Initiatives/MOOSE/ moose.pdf statement.org/Initiatives/MOOSE/moose.pdf
STROBE	Observational studies in epidemiology	http://www.strobe-statement.org
MOOSE	Meta-analyses of observational studies in epidemiology	http://www.consort- statement.org/Initiatives/MOOSE/ moose.pdf

*Statistics*: Whenever possible quantify findings and present them with appropriate indicators of measurement error or uncertainty (such as confidence intervals). Authors should report losses to observation (such as, dropouts from a clinical trial). When data are summarized in the Results section, specify the statistical methods used to analyze them. Avoid non-technical uses of technical terms in statistics, such as 'random' (which implies a randomizing device), 'normal', 'significant', 'correlations', and 'sample'. Define statistical terms, abbreviations, and most symbols. Specify the computer software used. Use upper italics (*P* 0.048). For all *P* values include the exact value and not less than 0.05 or 0.001. Mean differences in continuous variables, proportions in categorical variables and relative risks including odds ratios and hazard ratios should be accompanied by their confidence intervals.

**Results:** Present your results in a logical sequence in the text, tables, and illustrations, giving the main or most important findings first. Do not repeat in the text all the data in the tables or illustrations; emphasize or summarize only important observations. Extra- or supplementary materials and technical detail can be placed in an appendix where it will be accessible but will not interrupt the flow of the text; alternatively, it can be published only in the electronic version of the journal.

When data are summarized in the Results section, give numeric results not only as derivatives (for example, percentages) but also as the absolute numbers from which the derivatives were calculated, and specify the statistical methods used to analyze them. Restrict tables and figures to those needed to explain the argument of the paper and to assess its support. Use graphs as an alternative to tables with many entries; do not duplicate data in graphs and tables. Where scientifically appropriate, analyses of the data by variables such as age and sex should be included.

**Discussion:** Include summary of key findings (primary outcome measures, secondary outcome measures, results as they relate to a prior hypothesis); Strengths and limitations of the study (study question, study design, data collection, analysis and interpretation); Interpretation and implications in the context of the totality of evidence (is there a systematic review to refer to, if not, could one be reasonably done here and now?, what this study adds to the available evidence, effects on patient care and health policy, possible mechanisms); Controversies raised by this study; and Future research directions (for this particular research collaboration, underlying mechanisms, clinical research). Do not repeat in detail data or other material given in the Introduction or the Results section. In particular, contributors should avoid making statements on economic benefits and costs unless their manuscript includes economic data and analyses. Avoid claiming priority and alluding to work that has not been completed. New hypotheses may be stated if needed, however they should be clearly labeled as such. About 30 references can be included. These articles generally should not have more than six authors.

#### **Review Articles:**

It is expected that these articles would be written by individuals who have done substantial work on the subject or are considered experts in the field. A short summary of the work done by the contributor(s) in the field of review should accompany the manuscript.

The prescribed word count is up to 3000 words excluding tables, references and abstract. The manuscript may have about 90 references. The manuscript should have an unstructured Abstract (250 words) representing an accurate summary of the article. The section titles would depend upon the topic reviewed. Authors submitting review article should include a section describing the methods used for locating, selecting, extracting, and synthesizing data. These methods should also be summarized in the abstract.

The journal expects the contributors to give post-publication updates on the subject of review. The update should be brief, covering the advances in the field after the publication of the article and should be sent as a letter to editor, as and when major development occurs in the field.

#### Case reports:

New, interesting and rare cases can be reported. They should be unique, describing a great diagnostic or therapeutic challenge and providing a learning point for the readers. Cases with clinical significance or implications will be given priority. These communications could be of up to 1000 words (excluding Abstract and references) and should have the following headings: Abstract (unstructured), Key-words, Introduction, Case report, Discussion, Reference, Tables and Legends in that order.

The manuscript could be of up to 1000 words (excluding references and abstract) and could be supported with up to 10 references. Case Reports could be authored by up to four authors.

#### Letter to the Editor:

These should be short and decisive observations. They should preferably be related to articles previously published in the Journal or views expressed in the journal. They should not be preliminary observations that need a later paper for validation. The letter could have up to 500 words and 5 references. It could be generally authored by not more than four authors.

#### Other:

Editorial, Guest Editorial, Commentary and Opinion are solicited by the editorial board.

**References** References should be *numbered consecutively* in the order in which they are first mentioned in the text (not in alphabetic order). Identify references in text, tables, and legends by Arabic numerals in superscript with square bracket after the punctuation marks. References cited only in tables or figure legends should be numbered in accordance with the sequence established by the first identification in the text of the particular table or figure. Use the style of the examples below, which are based on the formats used by the NLM in Index Medicus. The titles of journals should be abbreviated according to the style used in Index Medicus. Use complete name of the journal for non-indexed journals. Avoid using abstracts as references. Information from manuscripts submitted but not accepted should be cited in the text as "unpublished observations" with written permission from the source. Avoid citing a "personal communication" unless it provides essential information not available from a public source, in which case the name of the person and date of communication should be cited in parentheses in the text.

The commonly cited types of references are shown here, for other types of references such as newspaper items please refer to ICMJE Guidelines (http://www.icmje.org or http://www.nlm.nih.gov/bsd/uniform\_requirements.html).

#### Articles in Journals

1. Standard journal article (for up to six authors): Parija S C, Ravinder PT, Shariff M. Detection of hydatid antigen in the fluid samples from hydatid cysts by co-agglutination. Trans. R.Soc. Trop. Med. Hyg.1996; 90:255–256. 2. Standard journal article (for more than six authors): List the first six contributors followed by *et al.* 

Roddy P, Goiri J, Flevaud L, Palma PP, Morote S, Lima N. *et al.*, Field Evaluation of a Rapid Immunochromatographic Assay for Detection of *Trypanosoma cruzi* Infection by Use of Whole Blood. J. Clin. Microbiol. 2008; 46: 2022-2027.

3. Volume with supplement: Otranto D, Capelli G, Genchi C: Changing distribution patterns of canine vector borne diseases in Italy: leishmaniosis vs. dirofilariosis. *Parasites & Vectors* 2009; Suppl 1:S2.

#### Books and Other Monographs

1. Personal author(s): Parija SC. Textbook of Medical Parasitology. 3rd ed. All India Publishers and Distributors. 2008. 2. Editor(s), compiler(s) as author: Garcia LS, Filarial Nematodes In: Garcia LS (editor) Diagnostic Medical Parasitology ASM press Washington DC 2007: pp 319-356.

3. Chapter in a book: Nesheim M C. Ascariasis and human nutrition. *In* Ascariasis and its prevention and control, D. W. T. Crompton, M. C. Nesbemi, and Z. S. Pawlowski (eds.). Taylor and Francis,London, U.K.1989, pp. 87–100.

#### Electronic Sources as reference

Journal article on the Internet: Parija SC, Khairnar K. Detection of excretory *Entamoeba histolytica* DNA in the urine, and detection of *E. histolytica* DNA and lectin antigen in the liver abscess pus for the diagnosis of amoebic liver abscess .*BMC Microbiology* 2007, 7:41.doi:10.1186/1471-2180-7-41. http://www.biomedcentral.com/1471-2180/7/41

#### Tables

- Tables should be self-explanatory and should not duplicate textual material.
- Tables with more than 10 columns and 25 rows are not acceptable.
- Number tables, in Arabic numerals, consecutively in the order of their first citation in the text and supply a brief title for each.
- Place explanatory matter in footnotes, not in the heading.
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# **Clinical aspects of human Bocavirus 1 in a sample of Egyptian infants with acute lower respiratory tract infection: A pilot study** Tayseer M. El-Zayat<sup>a</sup>, Hala G. El-Nady<sup>b</sup>, Soheir I. Mohamed<sup>a</sup>, Amira S. El Refay<sup>b</sup>, Ahmed N. El Tawel<sup>c</sup>, Mona Gaber<sup>d</sup>, Mohamed A. Ali<sup>c</sup>

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#### Background/aim

The recent advance in molecular technology permitted a comprehensive range of novel viral etiological causes to be detected in respiratory tract specimens. The human Bocavirus 1 (HBoV1) as a virus of the Parvoviridae family recently was defined as a human pathogen mainly linked to acute respiratory infection in children. Our aim was to identify the rate and seasonal variation of Bocavirus 1 infection and their correlation with the different clinical presentations in Egyptian infants less than 2 years old with acute lower respiratory tract infection.

#### Patients and methods

This study enrolled 100 infants aged from 4 weeks to 2 years presenting with symptoms of acute respiratory infection from Materia Hospital and Alzahraa University Hospital. Full medical history, general and local examination of the chest focusing on respiratory rate and plain chest radiograph were collected from each infant. In addition, pharyngeal swabs were collected from participated infants and were subjected to DNA extraction followed by PCR using different viral protein-targeted primer sets.

#### Results

Bronchopneumonia was the prominent diagnosis in the enrolled infants (38%) followed by bronchiolitis (34%) and bronchitis (28%) subsequently. The presence of HBoV1 among studied patients was 8% and the peak of the infection was in January (37.5%) followed by April (25%) and July (25%). Most of patients with HBoV1 positive were presented with respiratory distress and refusal of feeding. Seven percent of all patients were presented with diarrhea.

### Conclusions

HBoV1 infection may be considered as a risk factor for lower respiratory tract infections in Egyptian children less than 2 years old.

#### Keywords:

Bocavirus, bronchopneumonia, infants, molecular

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# Introduction

Respiratory tract infections are considered one of the main causes of childhood deaths and illness all over the word, especially in young children, it considered one of the main public health problem and this is owes to the easy and rapid spread of respiratory infection among the community which lead to high incidence and prevalence [1]. According to WHO reports, more than 8% of mortality in the Eastern Mediterranean Region were contributed to respiratory tract infection [2] mainly for lower respiratory tract and the age between 1 month and 5 years was the most affected. Approximately 70% of deaths under 5 years due to respiratory infection were in children living in developing countries [3,4].

Human Bocavirus (HBoV) was initially recognized in 2005 as one of parvovirus family utilizing a DNase treatment protocol, random PCR amplification,

bioinformatics assessment, and high throughput sequencing [5]. HBoV1 is strongly implicated in causing lower respiratory tract infection, especially in young children, and several of the viruses such as and 4 have been HBoV2, 3, linked to gastroenteritis, although the full clinical role of this emerging infectious disease remains to be elucidated. By applying virus screening technique to samples obtained through naso-pharyngeal swabs and nasal wash from infected patient who were suffering from unresolved respiratory tract infections, it provided a 3.1% positive result rate for HBoV. Therefore, it was anticipated that HBoV1 is one of the important causative pathogen of respiratory tract diseases [5].

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HBoV1 has been identified in nasopharyngeal swabs and serum samples, stool, and urine samples collected from infants around 2 years old principally during winter. HBoV is often revealed in respiratory tract patients consisting of symptoms as infection sneezing, cough, rhinorrhea, and elevated body temperature [6]. Clinically, patients infected with HBoV1 are presenting by upper and lower infections bronchiolitis, respiratory tract as bronchopneumonia, bronchitis, and triggering of asthma exacerbation [7]. Clinical presentation usually continue for 1 or 2 weeks, but some cases reported association with prolonged fever [8]. HBoV is as well recognized in stool samples from children with diarrhea with percentage between 0.8 and 9% [9].

This study aimed to identify the frequency of infection and seasonal variation of HBoV1 in infants presenting with symptoms of acute lower respiratory tract infection and to correlate it with the different clinical presentations.

# Patients and methods Patients and study design

A pilot cross-sectional study included 100 Egyptian infants; aged from 4 weeks to 2 years; visiting the outpatient clinic and the inpatient Department at Alzahraa University Hospital and Mataria Teaching Hospital in Cairo; presenting with symptoms of acute respiratory infection. It was conducted all over 1 year from January to December 2020. The combination of signs and symptoms of lower respiratory tract infections including cough, fever, tachypnea (defined as rapid respiratory rate as 60 breaths each minute in children aged <2 months, >50 breaths each minute in children aged between 2 and 12 months, and >40 breaths each minute in children >12 months of age), poor feeding, irritability, lethargy, grunting, cyanosis. congenital Infants with any anomalies or immunological disorders who were presented with whooping cough were excluded.

# Ethics approval

The present study was conducted with the Code of Ethics of the World Medical Association, according to the principles expressed in the Declaration of Helsinki. The protocol of this survey was approved by the Medical Research Ethics Committee of the National Research Centre with approval number 16121. A written informed consent was taken from the parents or guardians of all children enrolled in the study (mothers/or fathers/or any caregivers).

### Data collection and clinical examination

Full medical history was collected from each infant joined the study and a structured questionnaire designed to fulfill the following data: history suggestive of lower respiratory tract infections including (fever, cough, difficulty of breathing, poor activity, irritability, and cyanosis) and symptoms suggestive of other system affection (gastrointestinal tract, renal, and neurological).

Detailed general and local examination of the chest focusing on respiratory rate, which was counted twice, and the average count was recorded. Signs of respiratory distress including tachypnea, working alae nasi and, subcostal and intercostal retraction were recorded.

A plain chest radiograph, posterior anterior view at the time of admission has evidence of hyperinflation, increase bronchovascular marking and heterogonous opacifications in both lung fields.

# Laboratory methods

Nasal swabs were collected in sterile vials from each infant, and all collected samples were subjected to DNA extraction followed by PCR using different viral protein-targeted primer sets to detect bocaviruses.

### Nasal swabs samples

Nasal swabs from participants were obtained using a mucus trap (ARGYLE DeLee; Kendall, Cambridge, Massachusetts, USA). The collected volume ranged from 0.5 to 1 ml. Viral transport media (Hank's Balanced Salt Solution; Gibco, Invitrogen, New York, New York, USA) with 2.5% w/v bovine serum albumin (Sigma, St Louis, Missouri, USA), 2% penicillin/streptomycin (Gibco, Invitrogen), and 2.5% HEPES buffer (Gibco, Invitrogen) was added to each aspirate. The nasal swabs were immediately placed at 4°C and transferred to the Center of Scientific Excellence for Influenza Viruses, National Research Center Giza, Egypt within 24 h and kept at -80°C for nucleic acid extraction and virus isolation as follows.

# **Extraction of DNA**

Viral DNA was extracted from  $140 \,\mu$ l of collected samples by using a viral DNA copurification kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol in class III biosafety cabinet. The extracted nucleic acid from each sample was aliquoted and kept at -20°C till the setup of the PCR reaction.

# Conventional PCR for detection of human Bocavirus 1 DNA

Using Phusion high fidelity PCR master mix kit (Thermo Scientific, waltham, USA) a 25  $\mu$ l reaction containing 12.5  $\mu$ l of 2× Phusion master mix, 1.5  $\mu$ l (10 pmol) specific forward primer HBoV188F 5'-GAGCTCTGTAAGTACTATTAC-3', 1.5  $\mu$ l (10 pmol) specific reverse primer HBoV542R 5'-CTCTGTGTTGACTGAATACAG-3' 7.5  $\mu$ l H<sub>2</sub>O and 2  $\mu$ l of extracted DNA [10]. The PCR cycling conditions were 98°C for 30 s then 40 cycles (98°C for 10 s, 53°C for 30 s, 72°C for 30 s), then 72°C for 10 min. PCR products were visualized by staining with ethidium bromide by agarose gel electrophoresis considering a positive PCR reaction when a band of the expected size of 354 base pairs for HBoV1.

### Statistical analysis

The collected data was coded, tabulated, and introduced to a PC using the Statistical Package for Social Science (SPSS 17; SOSS Inc.). All data were presented as number and percentage. The suitable analysis was done according to the type of data obtained for each parameter. Mann–Whitney test (U test) was used to assess the statistical significance of the difference of a nonparametric variable between two study groups.

# Results

The results reported in Table 1 shows that male patients comprised 53% while females comprised 47%. Their age ranged from 1.1 to 24 months and the mean was  $10.5\pm8.2$  months, 63 (63%) lives in urban areas and 37 (37%) lives in rural areas, 59% of patients were exposed to passive smoking.

Table 2 shows that most of the patients (93%) presented with cough and 81% were complaining of

Table 1 Demographic data of studied infants			
10.5±	8.2		
7 (1.1–	-24)		
Frequency	Percent		
47	47		
53	53		
63	63		
37	37		
84	84		
13	13		
3	3		
59	59		
41	41		
	f studied infants 10.5± 7 (1.1- Frequency 47 53 63 37 84 13 3 59 41		

dyspnea. Twenty-nine percent of patients were presented with diarrhea and 67% had a fair general appearance. Seventy-two percent of infants showed chest retractions. There was a decrease as air entry and fine Consenting crepitations in 38.8% of patients on auscultation. In addition, conventional PCR was used for detection of HBoV1 DNA and eight (8%) cases were positive for Bocavirus1, while the remaining 92% were negative among all the studied infants with acute lower respiratory infection (Fig. 1).

In the current study high incidence of Bocavirus1 infection in children under 2 years old was reported on January (37.5%) followed by April and July (each 25.0%), as shown in Table 3.

The present results indicated that there were 34% diagnosed with bronchiolitis, 28% with bronchitis and 38% of patients were diagnosed with bronchopneumonia. Bronchopneumonia was a slight common diagnosis among HBoV1 positive cases. However, there was insignificant difference between HBoV1 positive and negative groups according to clinical diagnosis as reported in Table 4.

Table 5 shows a slight predominance of females over males among HBoV1 positive cases and there was no statistically significant difference (P>0.05) in sex between positive and negative groups. The median age among the positive group was 10 months and there was no statistical difference between residence, mother age and passive smoking between positive and negative groups.

The present result exhibited that there was insignificant difference (P>0.05) between HBoV1 positive and negative groups regard medical history, using Mann–Whitney test. Most of the positive cases had prenatal complications. There was equality in the mode of delivery. Nearly all patients received vaccination (Table 6). Moreover, all positive cases gave the history of drinking tap water (not tabulated).

Table 7 showed that cough is a prominent feature among all HBoV1 positive cases, eight (100%) out of eight cases. Most of patients with HBoV1 positive were presented with respiratory distress and refusal of feeding. Seven percent of all patients were presented with diarrhea. Cyanosis was uncommon among positive cases. Most of cases of HBoV1 infection were associated with chest radiographs abnormalities (hyperinflation, increase bronchovascular marking, and heterogonous opacifications in both lung fields). There was no statistically significant difference (P>0.05)

Symptoms	N=100 [n (%)]
Cough	93 (93)
Dyspnea	89 (89)
Refusal of feeding	71 (71)
Irritability	45 (45)
Poor activity	22 (22)
Diarrhea	29 (29)
Signs	
General appearance	
Fair	66 (66)
	30 (30)
Toxic	4 (4)
Appearance	
Cyanosis	3 (3)
Pallor	31 (31)
Normal	66 (66)
Chest shape	
Normal	94 (94)
Deformity	6 (6)
Chest retractions	
Positive	72 (72)
Negative	28 (28)
Auscultation	
Decrease air entry and fine crepitation	38 (38)
Bilateral expiratory wheezes	33 (33)
Coarse crepitation and transmitted nasal sound	25 (25)
Normal	4 (4)
Investigations	
Abnormal chest radiograph	45 (45)
Normal chest radiograph	55 (55)
BOCA result	
Negative	92 (92)
Positive	8 (8)

Table 2 Frequency and percent	t of clinical presentation a	Ind Bocavirus 1	results among the studied infa	nts with acute lowe
respiratory infection				

Figure 1



Agarose gel electrophoresis pattern of amplified products (354 bp) by PCR. M: 100 bp DNA ladder; Lane 1: control positive (arrow); Lane 2: control negative, Lane: 3, 4, 5, 6, 7, 8, 9, 11, 12, 14, and 15: negative and Lane 10 and 13: positive Bocavirus 1 bands of 354 bp amplicon (arrow) (NB. loading volume of PCR product in controls is 5  $\mu$ l but in other samples are 20  $\mu$ l).

Table 3 Monthly distribution of human Bocavirus 1 in positive and negative groups of infants with acute lower respiratory infection

Monthly distribution	Boca positive ( <i>N</i> =8) [ <i>n</i> (%)]	Boca negative ( <i>N</i> =92) [ <i>n</i> (%)]	P value
January	3 (37.5)	10 (10.9)	0.031*
February	0	2 (2.2)	0.673
March	1 (12.5)	17 (18.5)	0.673
April	2 (25.0)	11 (12.0)	0.029*
Мау	0	20 (21.7)	0.140
June	0	10 (10.9)	0.325
July	2 (25.0)	5 (5.4)	0.037*
August	0	0	1.000
September	0	1 (1.1)	0.766
October	0	2 (2.2)	0.673
November	0	7 (7.6)	0.328
December	0	7 (7.6)	0.328
*Significant difference at <i>P</i> value less than 0.05 using Mann–Whitney test.			

between HBoV1 positive and negative groups according to clinical presentations using Mann–Whitney test.

## Discussion

HBoV1 recently was identified as a novel virus that is one of the Parvoviridae family that has a single linear positive sense or negative sense single stranded deoxyribonucleic acid genome. It was detected in nasopharyngeal lavage, sera and blood samples of young children with respiratory tract infections and in fecal samples of patients infected with gastro-enteritis [11].

Acute respiratory tract infection is one of the prevalent pediatric diseases especially under 5 years [12]. In our study the median age of Bocavirus positive infants was 10 months which agreed with other study that recorded

Table 4 Statistical comparison between human Bocavirus	1 positive and negative	e groups according to clinical diagnosis
--	-------------------------	--

Clinical diagnosis	Total N=100	HBoV1 negative [n (%)]	HBoV1 positive [n (%)]	P value
Bronchiolitis	34	32 (94.1)	2 (5.9)	0.754
Bronchitis	28	26 (92.9)	2 (7.1)	
Bronchopneumonia	38	34 (89.5)	4 (10.5)	

HBoV1, human Bocavirus 1. P value more than 0.05 is insignificant, using Mann-Whitney test.

Table 5 Statistical comparison	between human Bocavirus	1 positive and no	legative groups as r	egards demographic data
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	HBoV negative	HBoV positive	P value
Age/months [median (range)]	7 (1.1–24)	10 (1.2–24)	0.674
	n (%)	n (%)	
Sex			
Male	50 (94.3)	3 (5.7)	0.469
Female	42 (89.4)	5 (10.6)	
Residence			
Urban	59 (90.5)	6 (9.5)	0.794
Rural	33 (93.9)	2 (6.1)	
Passive smoking			
Yes	55 (91.7)	5 (8.3)	1.000
No	37 (92.5)	3 (7.5)	

HBoV, human Bocavirus. P value more than 0.05 is insignificant.

#### Table 6 Statistical comparison between human Bocavirus 1 positive and negative groups as regards medical history

Medical history	HBoV negative [n (%)]	HBoV positive [n (%)]	P value
Prenatal illness			
Negative	15 (88.2)	2 (11.8)	0.530
Positive	77 (92.8)	6 (7.2)	
Natal complication			
Normal	29 (87.9)	4 (12.1)	0.286
C/S	63 (94)	4 (6)	
NICU admission			
Yes	23 (92)	2 (8)	1.000
No	69 (92)	6 (8)	
Obligatory vaccination			
Positive	84 (92.3)	7 (7.7)	0.718
Negative	8 (88.9)	1 (11.1)	
Recurrent ALRTI			
Positive	32 (88.9)	4 (11.1)	0.390
Negative	60 (93.8)	4 (6.3)	

HBoV, human Bocavirus. P value more than 0.05 is insignificant, using Mann-Whitney test.

Clinical presentations	HBoV1 negative [n (%)]	HBoV1 positive [n (%)]	P value
Cough			
Yes	85 (91.4)	8 (8.6)	0.419
No	7 (100)	0	
Dyspnea			
Yes	83 (93.3)	6 (6.7)	0.187
No	9 (81.8)	2 (18.2)	
Refusal of feeding			
Yes	65 (91.5)	6 (8.5)	0.795
No	27 (92.9)	2 (7.1)	
Irritability			
Yes	42 (93.3)	3 (6.7)	0.727
No	50 (90.9)	5 (9.1)	
Poor activity			
Yes	21 (95.5)	1 (4.5)	0.499
No	71 (91)	7 (9)	
Diarrhea			
Yes	26 (89.7)	3 (10.3)	0.581
No	66 (93)	5 (7)	
Cyanosis			
Yes	3 (100)	0	1.000
No	89 (91.8)	8 (8.2)	
Radiograph			
Normal	8 (80)	2 (20)	0.446
Abnormal	40 (88.9)	5 (11.1)	

Table 7 Statistical comparison between human Bocavirus 1 positive and negative groups according to clinical presentations

HBoV, human Bocavirus. P value more than 0.05 is insignificant, using Mann-Whitney test.

the same age [13]. In contrast with other research which reported higher median age 14 months and this can be explained by different sample size [14].

The higher rate of lower respiratory tract infection among infants may be ascribed to immature immunity, and due to the fact that the children usually exposed to a greater load of viruses which associated with living circumstances such as crowding. Moreover, parental anxiety is more for the young infants leading to early health care consultation in the course of disease [14–16].

In our study males with respiratory tract infection were 53% while females were 47%. The slight predominance of males over females among children with respiratory illness was endorsed by other researchers [13] who represented 57.4, 54.5% males, respectively, among their studied cases. The higher rate of respiratory tract infection among males due to biological variations between males and females primarily due to sex hormones. Hormones like steroids is responsible for the arrangement of actions through the stimulation of the immune system. The X chromosome is partially controlling the hyperresponsiveness of the immune system in females. As females have two X chromosomes, inherited one chromosome from each parent, while males have one X chromosome inherited from the mother and one Y chromosome from the father. To prevent paired quantity of proteins in females, one of the X chromosomes is at random silenced during X chromosome inactivation that happens in the earliest embryogenesis in females. The X chromosome inactivation result in a female, around half of the cells express genes resulting from the mother X chromosome and the other half express genes from the father X chromosome. Therefore, detrimental alterations that appear in an X chromosome-related gene will end in protein loss in all cells in a male but in only half of cells in a female. The X chromosome has various genes which, directly or indirectly, are implicated in immunity [17,18].

In contrast, some researcher reported no significant difference between males and females [19]. Moreover, females were more susceptible to respiratory infection in other researches [20]. According to HBoV1 positive cases we found that the females are more susceptible to infection.

A percentage of 63% of our respiratory infection cases were urban populations while 37% were living in rural areas. This is in agreement with other researcher who conducted a study aimed to identify the prevalence of respiratory tract in Egypt and reported that children living in urban areas had a higher rate of respiratory infection events than children living in rural areas which is assumed to be linked to distance from medical facilities [21]. In contrast to our finding the rate of viral infection was higher in rural patients than urban patients in some research, we reported that the rate of viral infection was higher in urban patients than rural patients [22].

Bronchopneumonia was the predominant clinical diagnosis in the present study (38%), while 34% of patients were diagnosed with bronchiolitis. Clinical diagnosis was variant in other researches between bronchiolitis and pneumonia [23] but the predominant was bronchiolitis as it was reported in 75 and 27% of cases in previous researches [24–26].

Multiplex PCR helped different labs to identify a group of viruses concurrently while timesaving and with more analytical sensitivity. There are numerous complex assays assessed for respiratory viruses' identification [27].

As HBoV first identified in 2005, it has been discovered commonly all over the world in respirational samples, with a prevalence between 5 to 19% [28,29]. In the present study, HBoV1 was detected in eight (8%) cases of 100 nasal swabs. Although, we found lower percentage than two previous studies in Egyptian studies [30,31] who reported that HBoV1 was discovered in 56.8 and 22%, correspondingly, as well the results of other studies which found a 17.7% [32]. This wide range of percentage is contributed to the different age groups, using different diagnostic methods, different study periods and different sample size.

Previously some studies had detected HBoV1 DNA by PCR methods in nasal and pharyngeal samples between 5.7 and 7% in children with respiratory tract infection [6,33,34]. This variation is assumed because of climates changes and several factors affecting each country's respiratory infections prevalence. In our study, the HBoV1 infections peak happened through January 3/8 (37.5%) followed by April and July (25%) and least incidence in March 1/8 (12.5%), similar finding had been reported by other studies [31,35] which reported that HBoV positive cases occurred during late fall and winter.

Our results revealed that patients age ranged from 1.1 to 24 month with mean age  $10.5\pm8.2$  months which agreed with Meligy *et al.* [24] who was work on detection of viral respiratory tract infection in infant by PCR and found that age ranged from 1 to 30 months and mean  $9\pm7.92$  months. The higher rate of lower respiratory tract infection among infants and young children can be explained by underdeveloped

immune system, and a higher load of the infectious agent associated with living conditions such as crowding. Furthermore, the parents of younger children may seek healthcare earlier in the course of disease due to parental anxiety [36,37]. In our study the median age of positive cases of HBoV1 was 10 months which agreed with other studies [23,38].

Regarding dietetic history HBoV1 was detected 4/8 (8%) in bottle fed infants and the infection rate of HBoV1 between different patterns of feeding was statistically insignificant (P>0.05) which were in accordance with previous studies [22].

Concerning the clinical presentation of HBoV1 positive cases, all positive cases presented with cough which agreed with previous studies who reported cough in 69% up to 97.9% of patients [31,38] reported cough in 69.9% of patients and. Our results demonstrated that there were no significant statistical differences between positive and negative groups as regard tachypnea, dyspnea, and refusal of feeding. On contrary, in the same research [38] authors reported that patients with high virus load were more likely the patients who presented by tachypnea, wheeze, and or dyspnea.

In the current study, we found that comparison between negative and positive groups as regards sex shows no statistically significant difference and female were more than males in positive group. This is in agreement with a study carried out in Saudi Arabia by Abdel-Moneim *et al.* [39], where they found slightly more females were infected compared to males, and Bharaj *et al.* [40] and EL-Mosallamy *et al.* [22], where both males and females were equally affected by HBoV1. In UK, studies reported that the distribution of HBoV among females and males was not significantly different from the distribution of females and males in the entire cohort which was 53 and 47%, respectively [41,42].

As regards residence, 63% of the studied cases were urban inhabitants while 37% were rural inhabitants. This result is in agreement with the study by other study [43] in which the prevalence of respiratory tract infection in Egyptian children was investigated and it was reported that urban children had a higher rate of respiratory episodes than rural children which is owed to the limited health services and hospital facilities. In our study, the rate of viral infection was higher in urban patients than rural patients in contrast to EL-Mosallamy *et al.* [22] who found that the rate of viral infection was higher in rural patients than urban patients. While, by comparison HBoV1 positive and negative cases regarding our study did not report any significant difference and this can be explained by the small positive cases.

It is well studied that exposure to environmental tobacco smoke is an important modifiable risk factor for child hospitalization due to respiratory tract infection [44] but still results are conflicting. Our results revealed that 59% of studied infants were exposed to passive smoking [45]. Some studies confirmed that exposure to passive smoking, in particular maternal smoking, causes a statistically significant increase in the risk of developing lower respiratory infections in the first 2 years of life which did not agree with other studies who failed to report an association between the presence of smokers in the house and severe respiratory tract infection [46,47]. This is in agreement with our study as we did not report any significant difference between HBoV1 positive and negative cases.

Diarrhea was detected in positive cases in a percentage of 10.3% from all cases in our study, this is in contrast to a study which was conducted on children present by gastroenteritis [33] where diarrhea observed in 100% of cases. This discrepancy is owed to different study designs and type of patient. In our cases, although we studied a respiratory infection case only and we did not study diarrheic patients, but it was documented previously by other researcher that there is a positive correlation between detection of HBoV and diagnosis of respiratory disease in diarrheic children [48].

Regarding clinical presentation of HBoV positive cases, all positive cases presented with cough which agreed with Jiang *et al.* [38], who reported cough in 97.9% of patients. Our results showed that insignificant statistical differences were reported between positive and negative groups as regard tachypnea, dyspnea, and refusal of feeding. On contrary, it was reported the patients who had wheeze and tachypnea/dyspnea at presentation were more strongly related to the patients with high virus load [38].

According to abnormal chest radiographs (hyperinflation, increase bronchovascular marking, and heterogonous opacifications in both lung fields), 11.1% of patients who showed abnormal radiograph were positive for HBoV1. On contrary, other researchers were reported that 47% of patients had an infiltrate in radiograph [49,50].

Although HBoV1 is mainly causing respiratory infection and is less common to cause gastroenteritis but the environmental source of infection was studied. Waterborne transmission was studied [51] and a high

incidence of HBoV1 in sewage samples was detected which delivered evidence of its rotation in the local population. Moreover, other study [52] found even though there is no suggestion of water-borne transmission for HBoV1, the significant presence in sewage waters contribute that HBoV1can be transmitted to other water environments. So, a possible role of water transmission in the HBoV1 circulation should not be ignored in the present study. In our study all positive cases gave the history of drinking house tab water, while the role of HBoV in respiratory or gastrointestinal infections remains to be completely explained, the risk of infection via polluted water should be taken into consideration.

### **Conclusion and recommendation**

The prevalence of HBoV1 was 8% among the Egyptian infants aged from 4 weeks to 2 years with acute lower respiratory tract infection. The peak of the infection was in winter. HBoV1 infection may be considered as a risk factor for respiratory tract infection in children less than 2 years old. However, a limitation of this study was that current results cannot be extrapolated to the entire population of Egyptian children as it included two hospitals and one government only in Egypt. Thus, we recommend further studies with a larger sample size on larger age scales and different governorates.

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# **Conflicts of interest**

There are no conflicts of interest.

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# Nutritional and psychosocial behavior during coronavirus disease 2019 pandemic among children and adolescents during the curfew in Saudi Arabia: A cross-sectional study

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#### Background/aim

The coronavirus disease 2019 pandemic has forced governments around the world to impose national curfew measures, which in turn have changed the dietary and lifestyle habits and psychosocial health of the world's population, especially children and adolescents. Thus, this study was conducted in an attempt to assess the impact of coronavirus disease 2019 pandemic on the nutritional and psychosocial behavior among children and adolescents during the curfew in Saudi Arabia.

#### Patients and methods

This cross-sectional analytical observational study was conducted through an online questionnaire using Google Forms with a total sample of 1000 Saudi children and adolescents aged from 8 to 19 years old. Chi-square was used to compare adolescents and children in terms of dietary habits and psychosocial changes. Multivariate logistic regression was used to predict the risk factors for depression, inattention, hyperactivity, and aggression using sociodemographic characteristics.

#### Results

In this study, a significant association was found between age groups and changes in dietary habits (P < 0.001) in children and adolescents during curfew. The frequency of depression (P < 0.001), inattention, and hyperactivity (P < 0.001) was significantly higher in the adolescents compared with the children, while aggressive behavior was significantly higher in the children (P=0.001) compared with adolescents during curfew.

#### Conclusion

This study reveals a significant disruption in both dietary habits and psychosocial health that occurred in adolescents and children during the curfew period. The findings underscore the need to develop comprehensive strategies aimed at supporting the nutritional and mental health needs of this group during these unprecedented times.

#### Keywords:

Adolescents, children, coronavirus disease 2019, curfew, dietary, psychosocial, Saudi Arabia

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### Introduction

The coronavirus disease 2019 (COVID-19) disease is a newly discovered infectious pandemic disease caused by the coronavirus [1]. Millions of cases have been recorded around the world related to this disease [2]. The COVID-19 pandemic has had a significant social, physical, and economic impact on families all over the world as it continues to spread [3]. There is a lack of knowledge about the risk factors that can lead to serious illnesses, and no specific treatment exists to prevent or cure the disease. As a result, CDC recommendations have emphasized maintaining good hygiene, social distancing, and only leaving the house, when necessary, in order to avoid contracting the virus [4].

In April 2020, nearly half of the world's population went into lockdown as their governments requested, they stay home in more than 90 countries or regions to

reduce the spread of the disease [5]. The Kingdom of Saudi Arabia has suspended all schools and universities, and work has become remote, except for emergency services, in accordance with international activities and to combat the spread of COVID-19 [6].

The COVID-19 pandemic and the Kingdom of Saudi Arabia's measures to stem its spread resulted in increased stress induced by the disruption of daily routines, as well as fear and anxiety about the disease's spread and its implications for people's finances, work, families, and personal lives. In comparison with adults, children and adolescents are

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more likely to suffer from negative nutritional and psychosocial consequences because of these curfew measures [7]. The impact on these age groups is determined by several risk factors, including developmental age, current educational status, having special needs, preexisting mental health conditions, being economically underprivileged, and the child or parent being quarantined due to the fear of contracting the virus [8].

Several international studies have been reviewed to produce information about dietary habits and lifestyle behaviors during the COVID-19 pandemic. However, it is important to note that only a limited number of studies were conducted in Saudi Arabia regarding this topic. Sleep disturbances affect children and adolescents, increasing their risk of cardiovascular disease, anxiety, and mood swings [9,10]. Insufficient sleep can affect attention span, emotional health, immune function, and academic performance [11,12]. Children aged 5-17 are recommended to have nine to eleven hours of uninterrupted sleep [13]. Besides, peer relationships, physical activity, and outdoor activities are becoming increasingly important to children and adolescents. Losing these activities leads to decreased physical activity and increased screen time. Furthermore, these variables have a direct impact on sleep duration, quality, and the regularity of sleep patterns [13]. Low sleep rates during curfew are associated with weight gain and the development of unhealthy habits [14].

Exercise is an indispensable component of the physical and mental health development of children and adolescents [3]. Staying at home for long periods during the curfew results in a rise in sedentary behaviors and increased screen time [15]. Obesity, type-2 diabetes, hypertension, and many other noncommunicable diseases are all linked to a lack of physical activity and excessive screen time [16].

The COVID-19 curfew has been associated with adverse mental health consequences for children and adolescents [7]. The stress they face due to the curfew affects their psychological health, educational performance, and developmental achievement [15]. More than 91% of the world's student population has been negatively impacted due to closing schools and activities [16]. Studies show that children exhibit more restlessness, sleep nightmares, less attention, and significant separation problems [17–19].

Adequate nutrition is thought to be a factor in good health, particularly in childhood and adolescence [19].

It is essential to acquire healthy eating behaviors in the stage of transition from childhood to adulthood because they concomitantly influence current health status [20]. The World Health Organization (WHO) has published that following a healthy diet during the period of isolation can help in the prevention and treatment of COVID-19 disease [21].

By the end of March 2020, more than 150 million children and adolescents around the world had been affected by the curfew [22]. Furthermore, hearing or reading about COVID-19 on social media on a regular basis can cause stress, which can lead to overeating and 'food cravings' [3]. Cravings for carbohydrates can reduce stress by secreting serotonin, which has a positive effect on mood [23]. However, there is a proportional relationship between food craving and glycemic index, which is linked to an increased risk of developing cardiovascular disease and being overweight [24,25].

The purpose of this study is to investigate the changes in nutritional and psychosocial behavior that occur in children and adolescents before and during the curfew period, Saudi Arabia, as well as to determine the effect of the COVID-19 curfew on the dietary habits, psychosocial behavior, and lifestyle behavior of children and adolescents. Also, the possible negative effect of the COVID-19 pandemic on these vulnerable age groups helps establish a base on which protective measures can be initiated to prevent this negative effect. Moreover, the study will raise awareness among parents about this effect.

# Patients and methods Patients and study design

This cross-sectional analytical observational study was conducted among Saudi children and adolescents aged from 8 to 19 years old to observe the changes in dietary patterns, eating behavior, physical activity, and psychosocial problems. The recruitment of subjects started on October 16<sup>th</sup>, 2020, and ended on January 17<sup>th</sup>, 2021, via an online survey created by the Google Forms web survey platform. The online survey was shared through social media (WhatsApp, Twitter, Telegram, etc.). Additionally, the participants who filled out the survey were asked to share the survey link to increase the number of study participants (snowball technique).

After filtering the participants using the exclusion criteria, all children and adolescents who met the inclusion criteria were enrolled. The inclusion criteria comprise Saudi children and adolescents aged 8–19 years old, while the exclusion criteria include participants suffering from diseases.

#### **Ethical approval**

Ethical approval for this study was obtained from the Ethical Committee of the College of Applied Medical Sciences at Taibah University, Al-Madinah Al-Munawara, Saudi Arabia, with approval number 2020/59/206 CLN. The digital consent form was obtained before the start of data collection.

# Sample size

The targeted sample size for this study was 1067 participants, as estimated by Epi Info (Epi Info, CDC, Atlanta), with a 95% confidence level, 50% expected changes in food intake, and a 3% margin of error [25]. A total of 1043 participants were recruited from various regions in Saudi Arabia. However, only 1000 children and adolescents were valid. People who suffer from diseases (n=26, 2.5%) and people with missing data (n=17, 1.6%) were excluded from the final analysis.

#### Data collection instrument (questionnaire)

Before the start of the questionnaire, the participants were given a brief description of the study and its aim. The questionnaire consisted of 20 questions and was divided into three main sections. It assessed a variety of lifestyle behaviors, such as dietary habits, physical activity, screen time, and sleep patterns, as well as sociodemographic factors and sources of stress during the curfew. All the questions were written in such a way that they could be answered directly in comparison with 'before' or 'during' the curfew period, and they were translated from English into Arabic and back-translated from Arabic into English by two bilingual experts.

The first section was about the sociodemographic characteristics, including participants' age, gender, parental educational level, parental employment status, family income, and the effect of the COVID-19 pandemic on the families' income.

The second section was about the changes in dietary and lifestyle practices before and during the curfew. The questions were adapted to include the main food groups on the healthy food palm of the Saudi Dietary Guidelines (cereals and bread, vegetables, fruits, milk and dairy products, meat, and legumes). Additional food items were included, including salty snacks, sugary drinks, sweets, fast food, and canned food. Moreover, questions about the changes in weight, appetite, and water consumption, as well as the changes in physical activity, sleep, and screen time hours, were included. The choices given were increased, reduced, or no alterations. The reliability of the adapted questionnaire was tested through a pilot before the survey was administered.

The final section was related to psychosocial behaviors before and during the curfew. The emotional and behavioral problems were assessed using the Pediatric Symptom Checklist-17 [26]. It is a psychosocial screen designed to identify hyperactivity, attention, emotional conduct, relational difficulties with peers, depression, and aggressive behavior. A total score of 15 or higher suggests the presence of significant behavioral or emotional problems. The PSC-17 subscales have obtained a reasonable agreement with validated and accepted instruments for internalizing (depression), externalizing (aggression), hyperactivity, and attention problems.

#### Statistical analysis

Data analysis was performed using Statistical Package for the Social Sciences, version 28. Frequency and percentages were used to display categorical variables. A  $\chi^2$ Chi-square test was used to test for the presence of any associations between categorical variables, which was applied to test the association between the age group and the following (dietary habits, lifestyle aspects, and psychosocial assessment). Multivariate logistic regression was performed to predict the risk factors for depression, inattention, hyperactivity, and aggression using sociodemographic characteristics. The level of significance was set at P valueless than 0.05.

#### Results

A total of 1000 children and adolescents aged between 8 and 19 years were included in the study. There were 56.1% adolescents and 43.9% children. Most of the participants were female (74.2%) (n=742). About 57.3% of mothers had an academic education, while 43.6% of fathers had an academic education. Most of the fathers (75.1%) are employed, while 20.2% of them are unemployed and 4.7% are not alive. About 52.5% of the mothers are unemployed, while 46.6% are employed, and 0.9% are not alive. Most of the 530 families (53%) had a monthly income of more than 10 000 SR. The family's financial situation became worse for 308 (30.8%) of the participants during the curfew in comparison with the time before the curfew period. The sociodemographic profile of the participants and their parents is described in Table 1.

Table 1	Sociodemographic chara	cteristics o	of one	thousand
Saudian	children and adolescents	;		

Demographical characteristics	N (%)
Sex of the child/adolescents	
Male	258 (25.8)
Female	742 (74.2)
Age group (in y)	
Child (8–12 years)	439 (43.90)
Adolescents (>12-19 years)	561 (56.10)
Education level of fathers	
High school or lower/diploma	428 (42.80)
Academic	436 (43.60)
Postgraduate	123 (12.30)
Other	13 (1.30)
Education level of mothers	
High school or lower/diploma	373 (37.30)
Academic	537 (53.70)
Postgraduate	64 (6.40)
Other	26 (2.60)
Occupation of fathers	
Does not work	202 (20.20)
Work	751 (75.10)
Not alive	47 (4.70)
Occupation of mothers	
Does not work	525 (52.50)
Work	466 (46.60)
Not alive	9 (0.90)
Monthly income of the family (Saudi riyals)	
No income	15 (1.50)
Less than 5000	149 (14.90)
From 5000 to 10 000	306 (30.60)
More than 10 000	530 (53.00)
Family's financial situation	
Not changed	622 (62.20)
Reduced	308 (30.80)
Increased	70 (7.00)

Table 2 demonstrates the comparison of dietary habits among children and adolescents before and during the curfew. Adolescents consumed more carbohydrates (P = 0.001), meats (P = 0.019), vegetables (P = 0.001), fruits (P = 0.006), legumes (P = 0.004), fast foods (P = -0.001), canned foods (P = 0.001), and water (P = 0.001) than children. On the other hand, children were found to consume more dairy products (P = 0.001), salty snacks (P = 0.006), and sweet foods (P = 0.001) than adolescents.

Table 3 illustrates the association between age groups and changes in lifestyle during the curfew. Sleeping hours in adolescents were significantly higher than those of children (60.1% vs. 46.7%) (P < 0.001). Reduced physical activity was observed in adolescents compared with children (61.1% vs. 46.7%) (P < 0.001). Weight loss was found to be higher in adolescents than in children (37.3% vs. 23.7%) (P < 0.001). About 70.8% of adolescents and 74.3% of children said they spent more time watching TV and using computers, tablets, and mobiles during the curfew than before.

Table 4 displays the association between psychiatric assessment and age groups. The frequency of depression, inattention, and hyperactivity was significantly higher in adolescents compared with children during the curfew (P < 0.001). In contrast, aggressive behavior was significantly higher in children (P < 0.001) than adolescents. The total PSC score was significantly higher in adolescents than children (P < 0.001).

Table 5 shows the multivariate logistic regression models for predicting depression, inattention, hyperactivity, and aggression using sociodemographic factors. Regarding depression, there was significant predictability across age groups (P <0.001). Compared with children, adolescents had a greater developing depression. chance of Significant predictability regarding gender was also seen, with exhibiting a decreased incidence of females depression. As for inattention and hyperactivity, only the age group variable was shown to be a significant risk factor (P < 0.001), in which adolescents had a higher risk for inattention and hyperactivity. As for aggression, the variable of age group was also shown to be a significant risk factor, with adolescents having a lower risk for aggression (odds ratio = 0.61).

## Discussion

Globally, the ongoing COVID-19 epidemic has had a significant negative influence on people's health. The pandemic may negatively affect mental health, dietary habits, and sleeping habits due to prolonged home confinement, financial worries, daily stressful events, violence, and overuse of social media during this critical period [27].

According to our knowledge, this is the first study to assess the effects of the curfew on dietary, psychosocial, and lifestyle behaviors among children and adolescents in Saudi Arabia during the COVID-19 pandemic. The findings from our study are like those found in a study conducted in Romania, Palestine, Verona, Italy, the North Africa region, and Poland, which assessed factors contributing to weight gain among children and adolescents during the curfew. Furthermore, the study found an increase in the consumption of carbohydrates, salty snacks, energy drinks, and soft drinks, as well as a notable increase in fast-food consumption (45.5%) [26–28]. Many people favor eating sugary and salty foods for snacking due to

Table 2 Comparison of	dietary habit	t changes amon	g children and	d adolescents (	during the	e coronavirus	disease 2	2019 curfew
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	Age gr		
Variables	Children (N=439)	Adolescents (N=561)	P-value
Changes in carbohydrate consumption			< 0.001*
Not changed	206 (46.9)	196 (34.9)	
Reduced	41 (9.3)	100 (17.8)	
Increased	192 (43.7)	265 (47.2)	
Changes in dairy product consumption			< 0.001*
Not changed	240 (54.7)	274 (48.8)	
Reduced	53 (12.1)	133 (23.7)	
Increased	146 (33.3)	154 (27.5)	
Changes in meats and alternative consumption			0.019*
Not changed	265 (60.4)	289 (51.5)	
Reduced	84 (19.1)	126 (22.5)	
Increased	90 (20.5)	146 (26)	
Changes in salty snack consumption			0.006*
Not changed	120 (27.3)	129 (23)	
Reduced	60 (13.7)	119 (21.2)	
Increased	259 (59)	313 (55.8)	
Changes in vegetable consumption	()	()	< 0.001*
Not changed	254 (57.9)	277 (49.4)	
Reduced	116 (26.4)	137 (24.4)	
Increased	69 (15.7)	147 (26.2)	
Changes in fruit consumption	,		0.006*
Not changed	253 (57.6)	270 (48.1)	0.000
Beduced	91 (20.7)	126 (22.5)	
Increased	95 (21.6)	165 (29.4)	
Changes in legume consumption	00 (21.0)	100 (2011)	0 004*
Not changed	286 (65 1)	317 (56 5)	0.001
Reduced	94 (21 4)	127 (22.6)	
Increased	59 (13.4)	117 (20.9)	
Changes in energy drinks and soft drink consumption	00 (10.4)	117 (20.0)	0 123
Not changed	176 (40 1)	190 (33.9)	0.120
Reduced	122 (27.8)	168 (29 9)	
Increased	141 (32 1)	203 (36 2)	
Changes in fast-food consumption	(02.1)	200 (00.2)	< 0.001*
Not changed	122 (27.8)	181 (32 3)	< 0.001
Beduced	185 (42 1)	125 (22 3)	
Increased	132 (30 1)	255 (45 5)	
Changes in canned food consumption	102 (00.1)	200 (40.0)	< 0.001*
Not changed	239 (54 4)	132 (23 5)	< 0.001
Beduced	123 (28)	244 (43 5)	
Increased	77 (17 5)	185 (33)	
Changes in sweet feed consumption	11 (11.5)	103 (00)	< 0.001*
Net changed	120 (21 7)	04E (42 Z)	< 0.001
Reduced	100 (22.8)	243 (43.7) 171 (20 E)	
headced	100 (22.0)	145 (25.8)	
Changes in water drinking	200 (43.0)	140 (20.0)	~ 0.001*
Not changed	076 (60 0)	240 (42 8)	< 0.001
Reduced	210 (02.9) 65 (11 9)	240 (42.0) 02 (16 6)	
	00 (14.0)	90 (10.0) 209 (40.6)	
Increased	98 (22.3)	228 (40.6)	

\*Significant at *P* valueless than 0.05, using  $\chi^2_{,}$  Chi-square test.

stress induced by the curfew, and this habit may increase the risk of developing obesity. In addition to that, screen time, sleeping hours, and physical activity among children and adolescents were all affected by the curfew [29]. The study revealed a substantial increase in the number of hours spent on screens and a decrease or lack of physical activity in comparison with before the curfew among children and adolescents. A study conducted in Canada explained the fact that children and

	Age groups [n (%)]		
Lifestyle aspect	Children (N=439)	Adolescents (N=561)	P value
Change in the number of hours spent on screen			0.228
Not changed	77 (17.5)	100 (17.8)	
Reduced	36 (8.2)	64 (11.4)	
Increased	326 (74.3)	397 (70.8)	
Change in the number of sleeping hours			< 0.001*
Not changed	162 (36.9)	118 (21)	
Reduced	72 (16.4)	106 (18.9)	
Increased	205 (46.7)	337 (60.1)	
Change in physical activity			< 0.001*
Not changed	169 (38.5)	191 (34)	
Reduced	205 (46.7)	343 (61.1)	
Increased	65 (14.8)	27 (4.8)	
Change in body weight			< 0.001*
No	175 (39.9%)	127 (22.6%)	
Reduced	104 (23.7%)	209 (37.3%)	
Increased	160 (36.4%)	225 (40.1%)	

Table 3 Comparison of mestyle changes among children and addrescents during the coronavirus disease 2019 d	) curfe
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\*Significant at *P* value less than 0.05, using  $\chi^2$ , Chi-square test.

adolescents attending school lessons, solving homework, and studying online on a smart phone or laptop have a lot of free time during the curfew period, which indicates spending more time on the screen [28].

In our present study, sleeping hours in adolescents were significantly higher than those in children (60.1% vs. 46.7%) (P<0.001). Moore et al. 2020 found that children slept for longer hours during the day (including naps) after the COVID-19 pandemic. Similarly, a study conducted on Italian children indicated that the participating children's sleep increased during the curfew by 6 h/day compared with the period before the curfew [14]. Another conducted children study on Chinese and adolescents reported that about 40% had trouble sleeping, staying asleep, or sleeping for a long period of time [30].

Considering the impact of COVID-19 and related consequences, this study revealed a higher rate of depression, the total PSC score, inattention, and hyperactivity in the adolescents' group as compared with children's group (P < 0.001) during the curfew. When comparing the results of this study to those of other studies conducted during the COVID-19 pandemic, depression was present in 51.7% of adolescents and 25.3% of children, which was lower than that of Lin et al., 2020, who studied 5641 Chinese individuals of various age groups [21]. Moreover, when the findings were compared with a study conducted in Spain, during the first weeks of COVID-19 curfew, large numbers of young students experienced a moderate score of depression [31]. In our present study, aggressive behavior was significantly higher in children's group (P=0.001) compared with the adolescents, which is consistent with an Egyptian

Table 4	Comparison of	psychosocial assessment amon	g children and adolescents du	ing the coronavirus disease 2019 curfew
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	Age group [n (%)]			
Psychosocial assessment	Children (N=439)	Adolescents (N=561)	P value	
Depression			< 0.001*	
Negative	328 (74.7)	271 (48.3)		
Positive	111 (25.3)	290 (51.7)		
Inattention and hyperactivity			< 0.001*	
Negative	377 (85.9)	353 (62.9)		
Positive	62 (14.1)	208 (37.1)		
Aggression			0.001*	
Negative	309 (70.4)	444 (79.1)		
Positive	130 (29.6)	117 (20.9)		
Total psychiatry assessment			< 0.001*	
Normal	285 (52.9)	254 (47.1)		
Abnormal	154 (33.4)	307 (66.6)		

\*Significant at *P* valueless than 0.05, using  $\chi^2$ , Chi-square test.

Factor	P value	Odds ratio		95% Confidence interval	
Multivariate regression model predicting	depression				
Age group (adolescent vs. child)	< 0.001*	3.70	2.73	5.01	
Sex (female vs. male)	0.042*	0.71	0.51	0.99	
Multivariate regression model predicting	inattention and hyperact	ivity			
Age group (adolescent vs. child)	< 0.001*	3.24	2.30	4.58	
Multivariate regression model predicting	aggression				
Age group (adolescent vs. child)	0.003*	0.61	0.45	0.85	

Table 5 Multivariate logistic regression	(predicting the risk	factors for depression,	inattention, and aggression using
sociodemographic factors)			

\*Significant at *P value* less than 0.05.

study conducted by Alamrawy *et al.* [32], who reported that children have a higher rate of aggression than adolescents.

Culture plays a role in the differences in the frequency disorders between different of age groups. Sociodemographic factors may play a part in how stress is expressed [33,34]. In our study, a multivariate logistic regression model was used to predict depression. It was shown that the adolescent and male genders had significant predictability for depression during the curfew. This result was inconsistent with Fiorenzato et al. [35], who stated that depression during COVID-19 curfew is more prevalent in females than males. Furthermore, she stated that females experienced a greater change in eating behaviors, more pronounced appetite, hypochondria, and anxiety compared with males during the curfew, whereas changes in sleeping patterns were equally affected in both genders [35]. However, in Saudi Arabia, males are at a higher risk for depression during the curfew. This can be explained by the fact that males in Saudi Arabia had more freedom before the curfew, and they did not experience staying at home for a long time before or working from home.

### **Conclusion and future direction**

Sudden lifestyle changes, dietary changes, and psychiatric morbidity are evident in adolescents and children during the COVID-19 pandemic. This study reveals a significant disruption in both dietary habits and psychosocial health that occurred in adolescents and children during the curfew period. The findings underscore the need to develop comprehensive strategies aimed at supporting the nutritional and mental health needs of this group during these unprecedented times. Implementing targeted interventions and strengthening flexible support systems becomes essential to mitigate the negative impacts experienced during periods of societal restrictions.

#### Recommendations

There is a significant need to raise awareness about healthy eating habits, the importance of home physical activity, and positive coping mechanisms to reduce the negative impact by promoting nutritional and physical activity programs in children's and adolescents' daily routines during the curfew. Finally, provisional and reliable nutrition information, as well as psychological support, is critical during the curfew period. Governments, health professionals, social media influencers, schools, and parents must all be aware of the COVID-19 curfew's implications.

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#### **Conflicts of interest**

There are no conflicts of interest.

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# Effect of interventional health educational program on clinical improvement in a sample of Egyptian school-age asthmatic children

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#### Background/aim

Asthma is one of the most widespread chronic disorders. To achieve better control over the disease, health intervention education programs could be applied to manage asthma and its consequences effectively to have a better quality of life. This study aimed to evaluate the impact of interventional health education programs on asthma symptoms and the quality of life of Egyptian asthmatic children.

Patients and methods

This study was conducted as an interventional study on asthmatic children and patients aged 6-16 years. Potential asthmatics according to GINA guidelines were enrolled in the study. Pulmonary function tests were applied including forced expiratory volume in the first second (FEV1), forced vital capacity (FVC) and FEV1/FVC ratio. 97 asthmatic cases were evaluated by asthma scoring, asthma control, and pediatric asthma quality of life questionnaire. All the study procedures were conducted at the start of the study and after applying the educational intervention program.

#### Results

The present results reported marked significant improvement (P < 0.05) in the respiratory symptoms after the application of the intervention education program including cough, night symptoms, dyspnea, and chest pain. In addition significant improvement (P < 0.05) occurred in the posteducational pulmonary function tests, including FEV1, prebronchodilator and postbronchodilators while there is no significant difference pre and postbronchodilator, FVC compared with the preeducational pulmonary function tests.

#### Conclusion

The interventional health education program has been useful for the improvement of clinical symptoms, pulmonary function, and quality of life of Egyptian school-age asthmatic patients and the performance of their families.

#### Keywords:

asthma, children, health education, intervention, pulmonary function tests

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# Introduction

Asthma is a troublesome childhood chronic disease. Although the effort done by the Global Initiative for Asthma to improve awareness about asthma burden and educate families about efficient management, asthma control is still suboptimal in several countries [1].

Asthmatic children typically describe a history of subsequent episodes of wheezing and/or cough initiated by upper respiratory infection, physical or weather changes. Wheezing and activity, coughing during physical activity or during laughing or crying, and episodes triggered in the absence of infection indicate asthma [2]. Most asthmatic children have constant disease, and only a few children face exacerbations requiring hospital or emergency room admission [3].

Poor asthma control is significantly associated with limitation of physical activity, school absence, emergency unit stay in, and hospital admissions [4]. Then, inappropriate asthma management adversely influences the quality of life of children with asthma and induce parental stress [5].

Studies have stated that poor asthma control may contribute to many factors as half of patients with chronic condition either do not take their medication probably or do not understand their condition satisfactory [2] leading to inadequate

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treatment compliance [6]. Asthma therapy main aim is to preserve children 'symptom free' by preventing chronic symptoms, preserving lung function, and permitting for daily normal activities [2].

Asthma children besides their caregivers need education programs to know more about their condition, the management plan, triggers of asthma, and home symptoms management. It is essential to improve public awareness, to decrease the myths and stigma related to asthma in some locations [7].

Asthma guidelines outline including national and international, suggested actions for children suffering from persistent asthma to enhance protective treatment and ensure optimum control of asthma [8]. These measures need committed engagement of a parents to distinguish relevant symptoms, reach and communicate with health care a provider to achieve an asthma action plan [9].

The present study aimed to evaluate the impact of interventional health education programs on the outcomes of treatment of asthma including clinical condition, pulmonary function and quality of life of Egyptian asthmatic school age patients and their caregivers.

# Patients and methods Patients

An interventional study included 97 asthmatic school age patients 6–16 years old, selected from primary and preparatory schools in Giza Governorate, Egypt, during the second part of the project titled 'Effectiveness of health education program on pulmonary functions and quality of life in Egyptian asthmatic children' and supported by the National Research Centre, Cairo, Egypt, from 2020 to 2021, in which 128 potential asthmatic cases were enrolled in the project.

All participants were interviewed and given appointments to be checked. Known asthmatic and probable cases (with positive family history of atopy, history of allergy and recurrent attack of respiratory tract infections and fulfilled the diagnostic criteria of bronchial asthma) were selected from medical records of schools with the assistance of medical staff schools (school doctors and nurses) and were included in the study. All the following cases were excluded: Child with an acute illness including upper and/or lower respiratory tract infections or child have congenital heart disease, known immunodeficiency, rheumatologic disease, cystic fibrosis, renal disease or congenital anomaly, history of receiving systemic steroids for disorder other than asthma and children with mental disability.

# Study design

A quasi experimental design was applied for this study (pre and post-test). In medical informatics, the quasiexperimental design, is often described as nonrandomized, pre and postintervention research, which often used to evaluate the influences of certain interventions. The increasing health care organizations capacity to obtain routine clinical data has led to the growing use of quasi experimental study designs in the field of medical informatics as well as in other medical disciplines [10].

# Ethical approval

The current study was conducted along with the Code of Ethics of the World Medical Association, agreeing to the principles expressed in the Declaration of Helsinki. The current study has been approved by the local Ethics Committee of National Research Centre, Cairo, Egypt with approval number 16/381. The caregiver or legal guardian of each participating child provided written informed consent before their inclusion in the study.

### Study population

All the 128 subjects enrolled in the project were invited to the health education program. Only 97 patients and their families approved to complete the second part of the study. Eligible subjects who gave approval were telephoned to verify eligibility and schedule the first date of the sessions. The program was held on subsequent six sessions in which the asthmatic children and their caregivers were invited to active participation.

### Educational program

Sessions were held in the Medical Research Centre of Excellency as an interactive workshops at baseline and 2nd, 3rd 4th, and 5th months by a collaborative team, Expert professors from Child Health Department gave the theoretical lecture and the team of pediatric researchers helped as facilitators to cover the applied part and make sure of the communication between all the participations.

During the session, the following topics were discussed with the asthmatic children and their caregiver (asthma facts, how to prevent asthma episodes, controlling the asthma symptoms and triggers, using medical devices and inhalers, common mistakes and pitfalls in asthma home management including common myths and diet appropriate to asthma patients) the scientific source of these topics were collected and prepared by the project team in a simple Arabic language as a power point and handouts for the participants.

#### Asthma and quality of life

All the asthmatic children were subjected to asthma scoring, Pediatric asthma quality of life questionnaire (PAQLQ) and The Pediatric Asthma Caregiver's Quality of Life (PACQLQ) pre and post educational program [11,12] were done for all caregivers.

The primary target was Asthma control as the studied cases answered the seven-asthma control questionnaire (ACQ) concerning symptoms, activity limitation, bronchodilator use and airway caliber by spirometer, each question was scored and then the total score was calculated according to the manual [13].

The score greater than or equal to 1.15 was considered positive=poor asthma control and the score less than 1.15 was considered negative=well asthma control.

Caregiver reports all the hospital visits and compliance to treatment over the 6 months to assess the quality of life of the children [12].

Caregiver health-related quality of life was evaluated using the Pediatric Asthma Caregiver's Quality of Life (PACQLQ). The 13 questions evaluate the difficulties with limitation of physical activity, emotional behaving, and a total score faced by parents of children with asthma, with better scores suggesting a better parental health-related quality [11].

### **Pulmonary functions test**

Pulmonary function test was applied to the entire participant pre-educational as a part of the base line enrollment to prove the asthma and asses the degree of severity according to GINA guidelines 2017. Then Pulmonary function tests were applied to all the 97 children and adolescents who completed the study. This was held in the pediatric pulmonary function unit in the Medical Centre of Excellency in The National Research Centre using the MS Pediatric unit (Erich Jaeger Gmbh-Hochberg, Germany). Flow-volume curves were performed according to American Thoracic Society standards and Impulse Sociometry (IOS) for the sociometric determination of respiratory impedance.

#### Sample size

The sample size was calculated using Sample power 3. It was based on the mean and differences in FEV1 and

FEV1% pre and post intervention 10. With 80% power (beta error=0.2) and an alfa level set at 0.05, a minimum of 40 participants are required [10].

#### Statistical analysis

The collected data were coded, tabulated and statistically analyzed using IBM SPSS statistics (Statistical Package for Social Sciences) software version 28.0, IBM Corp., Chicago, USA, 2021. Quantitative data was tested for normality using Shapiro–Wilk test, then described as mean±SD, and then compared using independent *t*-test (two independent groups) and paired *t*-test (paired data). Qualitative data were illustrated as number and percentage and was compared using  $\chi^2$  test. The level of statistical significance for all tests was set at *P* less than 0.05, otherwise the tests were considered insignificant.

# Results

Descriptive data are illustrated in (Table 1). Among the studied group 54 (55.7) were female and 43 (44.3) were males. A percentage of (45.4%) had positive history of allergy and regarding the potential exposure to smoking, it was showed that 79 (81.4%) cases reported positive exposure, which are significantly difference (P<0.001) than nonexposure to smoking (18.6%). Concerning the severity of asthma, the enrolled children gave history to mild (28.9%) and moderate persistent asthma 71.1%. Among all the participant children 44 (45%) were controlled.

The frequency of respiratory symptoms in the studied cases pre and post intervention education program

Table 1	Characteristic data of asth	matic cases under study	
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Age in years <sup>#</sup>	10.4±3.5 No (%)	$\chi^2$	P value
Sex			
Male	43 (44.3)	0.83	0.83
Female	54 (55.7)		
History of allergy			
Yes	44 (45.4)	0.51	0.475
No	53 (54.6)		
Potential exposures to sn	noke		
Yes	79 (81.4)	38.36	< 0.001*
No	18 (18.6)		
Severity of asthma			
Mild persistent	28 (28.9)	15.68	< 0.001*
Moderate persistent	69 (71.1)		
Control of asthma			
Controlled	44 (45.4)	0.51	0.475
Not controlled	53 (54.6)		

<sup>#</sup>: Data presented as Mean±SD (n=97 cases). <sup>••</sup>Correspondence to : Significant difference at *P* value less than 0.05, using  $\chi^2$  test).
Table 2	Frequency of	of respiratory	symptoms i	n the studied	cases pre	and postintervention	n education program	n application
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Respiratory symptoms	Pre educational No (%)	Post educational. No (%)	$\chi^2$	P value
Cough				
1 dry	55 (56.7)	30 (30.9)	13.087	0.001*
2 productive	50 (51.5)	27 (27.8)	11.392	0.001*
Timing				
1 morning	5 (5.2)	4 (4.1)	0.116	0.733
2 nights	78 (80.4)	28 (28.9)	51.994	0.001*
3 all day	43 (44.3)	22 (22.7)	10.203	0.001*
Respiratory difficulty				
1 tachypnea	17 (17.5)	8 (8.2)	3.719	0.054
2 dyspnea	70 (72.2)	34 (35.1)	26.862	0.001*
3 grunting	10 (10.3)	4 (4.1)	2.771	0.096
Wheezing	65 (67.0)	51 (52.6)	4.203	0.040*
Hoarseness of Voice and/or Stridor	44 (45.4)	23 (23.7)	10.055	0.002*
Chest pain	44 (54.4)	20 (20.6)	13.431	0.001*
Chocking	15 (15.5)	7 (7.2)	3.281	0.070
Cyanosis	19 (19.6)	6 (6.2)	7.76	0.005*

<sup>\*</sup>: Significant difference at *P* value less than 0.05, using Chi square test ( $\chi^2$ ).

application was reported in (Table 2). Marked statistically significant improvement in the respiratory symptoms had occurred after application of the intervention education program. 55 (56.7%) were complaining of dry cough after intervention percentage decreased to 30 (30.9%) with significant P value=0.001 night symptoms improved it was 78 (80.4) and became 28 (28.9) chest pain was improved at P value=0.001.

The data represented in (Table 3) showed, Pre and post education intervention program quality of life scores of asthmatic children and their care givers. It was found that a significant improvement (P<0.05) in post education quality of life scores of the children that including the activity limitation, symptoms, emotional function and mean total child score, compared with their pre-educational scores. Moreover a significant improvement (P<0.05) in their care givers quality of life scores with P value=0.001, using Paired sample t-test.

The data represented in (Table 4) showed, Pre and posteducation intervention program pulmonary

Table 3	Pre and post education	intervention program	quality of life scores of	of asthmatic children	and their caregivers
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•			•	
Quality of life scores	Pre educational	Post educational	t	P value
Child quality of life scores:				
Activity limitation score	4.51±1.23	5.82±1.03	6.918	0.001*
Symptoms score.	4.51±1.14	5.35±1.12		
Emotional function score	4.51±1.32	5.93±1.37		
Mean total child score	4.51±1.35	5.71±1.31		
Caregivers quality of life scores:				
Activity limitation score	5.14±1.65	6.17±1.32	6.701	0.001*
Emotional effect score	4.76±1.42	5.92±1.46		
Mean total care giver score	4.92±1.54	5.81±1.38		

\*: Significant difference at *P* value less than 0.05, using Paired sample *t*-test.

<b>Fable 4</b> Pre and post education intervention program	n pulmonary functions scores of asthmatic children
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Pulmonary functions	Pre educational	Post educational	<i>t</i> -test	P value
Pre bronchodilator FVC	90.70±9.078	92.61±7.082	1.801	0.085
Pre bronchodilator FEV <sub>1</sub>	83.70±11.534	88.70±5.363	-3.501	0.004*
Pre bronchodilator FEV <sub>1</sub> /FVC%	91.78±8.801	94.74±4.454	-2.915	0.0097*
P ost bronchodilator FVC	92.65±5.556	93.04±5.740	0.349	0.730
post bronchodilator FEV1	85.83±8.569	90.35±8.348	-5.589	0.0025*
post bronchodilator FEV <sub>1</sub> /FVC%	92.04±7.252	97.13±6.174	-6.124	0.0018*

FEV1, forced expiratory volume in the first second; FVC, forced vital capacity. \*: Significant difference at *P* value less than 0.05, using independent *t*-test.

functions scores of asthmatic children. A significant improvement (P<0.05) was reported only in FEV1, pre bronchodilator and FEV1 postbronchodilators while there is no significant difference in pre and post bronchodilator FVC.

# Discussion

Asthmatic children education in self-management of asthma has appeared as one of the therapeutic intervention to improve children to understand and control asthma, and positively to become active, selfefficacious asthma care participants [14]. Children usually depend on their caregiver for management of asthma. Therefore, asthma education should focus on whole family. This interventional evaluated the effectiveness of health education programs on the quality of life of asthmatic children and their caregivers.

By comparing respiratory symptoms frequency as before and after implementation of the educational program, there is significant improvement of symptoms was detected in asthmatic patients including cough, night symptoms, dyspnea and chest pain. This agreed with many previous studies [15–18]. Although these studies used different tools or variant sample size, but all the studies gave a positive change in asthma control after health education including less exacerbations, infrequent symptoms.

Asthma is often worse at night, however, asthma night symptoms as shortness of breath, wheezing, and cough be associated with sleep disorder are common which in turn lead to day somnolence, school absences, and poor quality of life. Few studies investigate the correlation between nocturnal asthma symptoms and quality of life [19,20]. In our study, there was a significant decrease in night cough after health education P less than 0.01.

Recurrent exacerbation, chest pain and cyanosis are considered an emergency condition which often needs an emergency department visit or even hospitalization. This led to more emotional and financial burden on the family [21,22].

In our study there was a significant difference in comparing pre and post education as regards these symptoms. This is in agreement with an Egyptian study reported less ER visits after health education program for asthmatic patients [23]. Similarly, a study conducted in Saudi Arabia on asthmatic children, found statistically significant decrease in ED visits, hospitalizations, and ICU admissions in the posteducation groups [24]. Also, another study proposed that asthma education was accompanied by 54% lower hospitalization risk and 31% lower emergency department visit risk than usual care. Sensitivity analysis found that the group that received health education had experienced a decrease clinic visit risk. This may be explained by the fact that asthma was better controlled after an educational intervention [25].

In our study there was a significant difference in comparing pre and post education as regard these symptoms. This can be contributed to many factors first; early warning signs can be identified by the patient or the care giver after proper health education and a correct performance in case of acute exacerbation. Second is the fact that many patients may not have the proper knowledge and skills of using different lines of treatment, especially spacer or inhalers which may need training from the health care provider. In our study we noticed that most of the children did not know the appropriate way to use inhalers. Moreover, many children did not know the importance of using spacer [26,27] and finally, as any chronic disease the compliance and adherence to the treatment affect the severity of the disease [28].

To sum up, all these findings support the theory that health education will lead to better asthma control and in turn better quality of life for both the patient and caregiver. In the current study significant improvement in quality of life scores of the children and their care givers was detected comparing pre and post educational scores. Many studies showed an improvement of quality of life after health education [23,29].

However, some studies showed that group education targeted to asthmatic children improved morbidity and understanding of the disease but not quality of life [30,31].

This can be explained by the applying different approach in health education. We used a face-toface interactive approach which have more learning impact over the remote way either by web based or online ways which overcome the traditional education system [32,33].

Clinical evaluation of asthmatic children and their follow up mainly depend on reliable information of lung function [34] therefore, In asthmatic children regular evaluation of pulmonary function is necessary to adjust management and ensure that management objectives are fulfilled [35]. Many studies have described the evaluation of lung function in asthmatic patients, as defined by monitoring forced expiratory volume in the first second FEV1, FVC, the FEV1/FVC ratio, and forced expiratory flow between 25 and 75% of the vital capacity (FEF25–75%), [34,36–38]. Some authors found an early decrease in lung function in 52% of the patients in asthmatic children suffering from moderate asthma [38].

By comparing the pulmonary function in asthmatic children in our study, a significant improvement was reported only in EFV1, pre bronchodilator and post bronchodilators while there is no significant difference post bronchodilator FVC. This was in agreement with Nunes C *et al.* [7] who observed that self-management education of asthmatic children enhances pulmonary function [39].

Other study conducted on children aged 8–12 years, stating that there was no statistically significant difference between pulmonary function regarding studied children within the data collection [31].

Although, Maximal expiratory flow volume curves are red the gold standard for the evaluation of pulmonary function in asthmatic children. In patient with poor asthma control symptoms, FEF25-75% can present airway obstruction better than can FEV1 and the FEV1/FVC ratio, both of which are often normal in children with asthma [34]. All children in our study were under treatment and more or less controlled. Even though pulmonary function test is an optimum way to monitor the asthmatic children, but it needs more periodic visits on more long-time scale. Periodic evaluation of pre and post bronchodilator FEV1 may be used to classify children at risk for developing a continuing decline in airflow. Epidemiologic research have reliably presented a tracing of FEV1 and FEV1/ (FVC) ratio from childhood to adulthood [40].

In our study, we found a significant improvement of pre and postbronchodilator FEV1 after educational program but there was no significant difference in pre and post bronchodilator FVC, while the present study reported significant improvement in post bronchodilator FEV1/FVC%. In young children, FEV1 may not be accurate and may have a different physiological significance as compared with older children. Moreover, on a shorter period, FEV1 has been presented to be an independent predictor of asthma attacks exacerbations: asthmatic children with a baseline FEV1 less than 60% predicted have a doubled asthma attacks risk in the following year as compared with children with an FEV1 greater than 80% predicted [35] FEV1 is essential in asthma severity scoring [41]. Though, most of school children have an FEV1 greater than 80% predicted, consequently within the known normal range independent of asthma severity when defined on symptoms base. Generally, both  $FEV_1$  and the  $FEV_1/FVC$  ratio relate weakly with symptom-based severity in children.

Asthmatic children who have poor awareness of bronchial obstruction may be at higher risk of severe asthma attacks and diminished pulmonary function which may lead to poor asthma outcomes [41] Thus, periodic evaluation of pulmonary function appears reasonable in observing of asthmatic children. Though, only 20–40% of primary health care providers suggest lung function assessment in asymptomatic children with asthma and up to 59% of clinician never recommend pulmonary function [42,43]. Spirometry is greatly recommended as part of the diagnostic work up of asthmatic children aged between 5 and 16 years old [44].

# Conclusion

In conclusion, quality of life of both asthmatic children and their families are affected significantly by asthma severity and its degree of control. Health educational programs should be conducted to Egyptian asthmatic children, their parents, and their caregivers because caregivers of asthmatic children suffer from many challenges and can be strongly affected by their child's disease, so the preventive measures, early diagnosis and the appropriate management of asthma should be well known for the asthmatic child caregiver.

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Author's contributions: H.G.E.N. was responsible for conceptualization, data curation, methodology, project administration, supervision, and review and editing, A.S.E.R., D.A.S. and H.A. were participated in the data curation, clinical management, investigation, visualization and writing the original draft, D.E.E., R.M.S. and D.M.E.M. were participated in data curation, formal analysis. All authors have read and approved the final manuscript.

### **Conflicts of interest**

There are no conflicts of interest.

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# Evaluation of eosinophil, immune-globulin E and Interleukin 6 levels in bronchial asthmatic children after management with needle and laser acupuncture

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### Background/aim

Bronchial asthma is a disabling disease causing interruption with the daily activities of children. Acupuncture proved to be beneficial in improving the symptoms of the disease and decreasing the dose of conventional medications that must be taken and may have serious side effects. This study aims to evaluate the variations of some inflammatory and immunity markers such as Eosinophil, immune-globulin E (IgE), and Interleukin 6 (IL6) in bronchial asthmatic children after treatment with needle and laser acupuncture.

### Patients and methods

In this randomized controlled clinical trial, we included 75 patients divided into three groups. Each group has 25 children. The patients in the first group received laser sessions three times a week for 4 weeks. Those in the second group were treated by needle acupuncture during the same period of time, while the children in third group received only conventional medications and were used as control. Complete blood cell count, which reveals eosinophilic count, was determined, as well as serum IgE and IL6 were measured using Elisa techniques at baseline and after one month of treatment.

### Results

All the patients in the three groups showed significant improvement concerning their symptoms and laboratory parameters number of attacks/week and night awakens/ month all decrease significantly (P<0.001) also IgE (P<0.001) after 1 month of treatment with needle or laser. However, children receiving needle and laser sessions showed more significant improvement (P<0.05) than the control group, with no differences between the two groups. White blood cells, eosinophils, IgE, IL 6 were statistically decreased (P<0.05) after the end of the sessions.

### Conclusion

Needle and laser acupuncture proved efficient in relieving the symptoms of the bronchial asthma in children more than conventional medications alone, as well as Eosinophil, IgE, and IL6 levels were significantly decreased after the treatment.

### Keywords:

acupuncture, asthma, eosinophil, immune-globulin E, Interleukin 6, laser

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# Introduction

Bronchial asthma, in childhood, is a disabling respiratory disease-causing interruption of daily child activities and affects the school and sports performance. Adequate and sufficient management of the disease enables the restoration of respiratory normal physiological performance. Acupuncture is a form of stimulation that modulates the activity of the autonomic nervous system [1,2].

Laser acupuncture has superiority over needle acupuncture in the fact that it is less invasive with easier acceptability for both parents and children. Bronchial hyper-responsiveness is affected by the autonomic nervous system interplay between sympathetic and parasympathetic system. Acupuncture causes neuro-modulation that enhances the bronchial tree smooth muscle relaxation. In addition, it causes immuno-modulation that would reduce the allergic response observed in asthma patients [3,4].

Management of asthma in childhood has been largely dependent on bronchodilators and mast cell stabilizers. However, intervention by acupuncture was observed by researchers all over the globe to reduce the medication doses and the immune pathological course of the disease [5,6].

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On the other hand, some viruses such as parainfluenza virus or Epstein-Barr virus, can cause triggering of immune-globulin E (IgE) antibodies and Eosinophil and interacting with the viral infection to exacerbate the acute asthma attacks, moreover, Th2 lymphocytes' are responsible to produce cytokines, such as Interleukin 6 (IL4) and IL-5 and IL 6 [6].

This study aims to evaluate the variations of some inflammatory and immunity markers such as Eosinophil, IgE, and IL6 in bronchial asthmatic children after treatment with needle and laser acupuncture.

# Patients and methods Patients

The current research study is a randomized clinical trial study, enrolled 75 asthmatic children aged from 12 to 16 years, males and females, and under conventional medical treatment, visiting the outpatient clinic of Allergy and Asthma Clinic, Pediatrics Hospital, Cairo University and the Laser and Acupuncture Clinic in the Centre of Excellence at National Research Centre, Cairo, from September to November 2023.

### Study design

Seventy five asthmatic children were divided randomly into three equal groups, as follows:

Group A: 25 children were subjected to laser acupuncture sessions, three times/week for 4 weeks with conventional medications.

Group B: 25 patients using needle acupuncture sessions for 1 month (three times/week) with conventional medications.

Group C: 25 patients were given conventional medical treatment only and were used as control group.

All Cases have been followed-up and assessed for 1 month.

### Inclusion criteria

According to GINA (2023) [7], the patients had partially controlled or uncontrolled asthma for at least 3 months and were diagnosed more than 6 months before the study. The peak expiratory flow rate (PEFR) was 60–80%, within one month before the application of the research.

# **Exclusion criteria**

The cases excluded from the study are those having heart failure, tuberculosis, and genetic disorders such as Down syndrome and cystic fibrosis.

### **Ethical consideration**

The present study was conducted with the Code of Ethics of the World Medical Association, according to the principles expressed in the Declaration of Helsinki. This study has been approved by the local Ethics Committee of National Research Centre, Cairo, Egypt with approval number 17 152, a written informed consent was provided by each participant before their inclusion in the study.

### Methods

All the cases had been subjected to history taking and full clinical examination IL 6, IgE, and leucocytic count were tested in all the patients before the therapy (baseline) and after one month (just after the last session).

### Laser device

Laser device used was diode (semiconductor laser), low power (cold laser). The machine used aculaser point with 100 mW power and 3000 HZ frequency. It has two probes, one shower that was not used in this study, the other one have tunable wave length ranging from 650 to 860 nm. In this study, we used the wavelength of 860 nm, the power of 100 mW for 20 s over each acupoint giving power density of 3 Joules/Cm<sup>2</sup>. (The laser wave was continuous) to stimulate acupuncture points: DU20 (BaiHui), BL23 (ShenShu), BL28 (Pang Guang Shu), SiShen Cong (3 cm from the point BaiHui), RN6 (QiHai), GB20 (FengChi) and Du16 (FengFu).

## Needle acupuncture

We used fine sterile disposable acupuncture needles  $(0.25 \times 25 \text{ mm})$  to stimulate acupuncture points: DU20 (BaiHui), BL23 (ShenShu), BL28 (PangGuangShu), SiShenCong (3 cm from the point BaiHui), RN6 (QiHai), GB20 (FengChi) and Du16 (FengFu), as shown in Fig. 1.

Sites of needle on skin of children had been sterilized on by alcohol swab before its application. Needles were entered till its <sup>1</sup>/<sub>4</sub> th of their length.

### Laboratory investigations

Complete blood count with differential white blood cell count, that reveals eosinophilic count, was determined using Blood Coulter of Mindari Co. (Germany).

Determination of Serum IgE and IL6 were performed by the enzymatic immunoassay method using the kits supplied from DRG International, Inc. (Springfield, NJ 07081, USA), according to manufacturer's instructions.



### Sample size calculation

It was depending on who found that the Mean±SD of VC% before and after LASER acupuncture were 62.7 ±14.2 and 75.2±12.3 respectively. Assuming that the correlation between before and after LASER acupuncture levels was 0.500, power= 0.80 and  $\alpha$ =0.05, and by using G\*Power 3.1.9.4 release the minimal sample size to detect a significant statistical change in VC% is 17 cases. We will recruit 25 cases for possible attrition, additional equal samples were recruited in the needle group and control group [8].

### Statistical methods

The collected data were coded, tabulated and statistically analyzed using IBM SPSS statistics (Statistical Package for Social Sciences) software version 28.0, IBM Corp., Chicago, USA, 2021. Quantitative data were tested for normality using Shapiro-Wilk test, then described as mean±SD and then compared using independent t-test (two independent groups) and ANOVA test (three independent groups), Bonferoni test were used for post hoc comparisons, as well as paired *t*-test (paired data). Qualitative data were described as number and percentage and compared using  $\chi^2$  test as well as Fisher's Exact test according to expected numbers in cells (independent groups), as well as marginal homogeneity test (paired ordinal data). The level of significance was taken at *P* value less than 0.05 was significant, otherwise was nonsignificant [9].

### Results

The data presented in Table 1 showed that insignificant statistical difference between the different asthmatic children groups treated with laser, needle, or control groups, regarding age, sex, weight, asthma severity, and antiasthma treatment.

Table 1	Demographic and	clinical history	between th	e study	groups
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Variables	Laser group (Group A)	Needle group (Group B)	Control group (Group C)	P value
Age (y)	13.6±1.3	13.2±1.2	13.5±1.3	^0.447
Sex				
Male	16 (64.0%)	16 (64.0%)	14 (56.0%)	#0.799
Female	9 (36.0%)	9 (36.0%)	11 (44.0%)	
Weight percentile	73.6±7.0	74.5±8.6	74.8±5.9	^0.817
Asthma severity				
Intermittent	4 (16.0%)	3 (12.0%)	3 (12.0%)	¤0.942
Mild persistent	4 (16.0%)	5 (20.0%)	7 (28.0%)	
Moderate persistent	13 (52.0%)	11 (44.0%)	10 (40.0%)	
Severe persistent	4 (16.0%)	6 (24.0%)	5 (20.0%)	
Treatment				
SABD	25 (100.0%)	25 (100.0%)	25 (100.0%)	NA
LABD	16 (64.0%)	14 (56.0%)	13 (52.0%)	#0.678
Montelukast	25 (100.0%)	25 (100.0%)	25 (100.0%)	NA

LABD, Long acting bronchodilators; NA, Not Applicable; SABD, Short acting bronchodilators. ^ANOVA test.  $\#\chi^2$ . Chi square test, #Fisher's Exact test.

Table 2 shows that insignificant statistical difference between the study groups regarding baseline number of attacks/week and number of night awakens/month. However, number of attacks/week and number of night awakens/month 1 month after treatment were significantly higher in control group (P<0.05) with no significant difference between the Laser and Needle groups. Reductions in number of attacks/week and number of night awakens/month were significantly lowest (P<0.05) in the control group with no significant difference between the Laser and Needle groups. The number of attacks/week and number of night awakens/month significantly decreased (P<0.05) than baseline, in all the study groups.

Table 3 showed that insignificant statistical difference between the study groups regarding baseline on hemoglobin, Leucocytes, Eosinophil, IgE, and IL-6.

One month after treatment, there was no significant difference between the study groups regarding the hemoglobin level and its change. One month after treatment were significantly highest (P < 0.05) in control group, while insignificant difference was reported between the Laser and Needle groups. Reductions in Leucocytes, Eosinophil, IgE, and IL-6 were significantly lowest (P < 0.05) in control group while insignificant difference was reported between the Laser and Needle groups. Hemoglobin exhibited insignificant changes in the study groups. Leucocytes, Eosinophil, IgE, and IL-6 showed significant decreases (P < 0.05) in all the study groups.

Table 4 showed that continued clinical improvement one month after medical treatment discontinuation significantly was significantly least frequent in

Table 2 Changes in the clinical condition between the study groups

Variables	Laser group (Total=25)	Needle group (Total=25)	Control group (Total=25)	^P value
Number of attac	ks per week			
Baseline	6.4±3.1	6.7±3.9	6.7±4.2	0.933
Month-1	1.8±2.7a	2.0±2.6a	4.3±4.0b	0.011*
<sup>§</sup> P value	<0.001*	<0.001*	<0.001*	
Number of night	awakens per month			
Baseline	7.4±4.6	8.6±3.9	8.0±6.4	0.693
Month-1	1.6±2.2a	2.6±2.2a	5.0±5.2b	0.003*
<sup>§</sup> P value	<0.001*	<0.001*	<0.001*	

All data are expressed as mean + SD. All data with different letters (a, b, c) are significant using ^ANOVA test. \*Significant difference using ^ANOVA or <sup>§</sup> Paired t-test at *P* less than 0.05.

Variables	LASER Group (Total=25)	Needle Group (Total=25)	Control Group (Total=25)	^P-value
Hemoglobin (gm	n/dL)			
Baseline	12.3±1.6	12.0±1.7	11.9±1.4	0.730
Month-1	12.3±1.5	12.1±1.7	11.7±1.4	0.295
<sup>§</sup> P value	0.581	0.357	0.054	
Leucocytes (x10	93/mL)			
Baseline	7.1±1.8	7.2±1.3	7.3±2.2	0.947
Month-1	5.7±2.0a	5.7±1.5a	7.0±2.1b	0.015*
<sup>§</sup> P value	<0.001*	<0.001*	0.038*	
Eosinophil (%)				
Baseline	13.7±2.9	13.6±3.8	13.8±2.7	0.975
Month-1	10.9±2.8a	10.8±3.2a	13.2±3.3b	0.010*
<sup>§</sup> P value	<0.001*	<0.001*	0.013*	
IgE (IU/mL)				
Baseline	1027.9±174.4	1005.8±157.3	1017.0±151.3	0.889
Month-1	248.1±119.2a	240.9±210.0a	514.4±173.1b	<0.001*
<sup>§</sup> P value	<0.001*	<0.001*	<0.001*	
ll-6 (pg/mL)				
Baseline	14.5±5.4	14.1±7.0	15.0±7.2	0.899
Month-1	7.2±3.9a	7.4±6.0a	13.2±7.4b	<0.001*
<sup>§</sup> P value	<0.001*	<0.001*	<0.001*	

All data are expressed as mean + SD. All data with different letters (a, b, c) are significant using ^ANOVA test. \*Significant difference using ^ANOVA or § Paired *t*-test at *P* less than 0.05.

Table 4	Improvement	one month afte	r medical treatment	discontinuation	between the st	udy cases
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Improvement	Laser group (Total=25)	Needle group (Total=25)	Control group (Total=25)	P value
Continued	19 (76.0%)a	18 (72.0%)a	9 (36.0%)b	#0.006*
Discontinued	6 (24.0%)	7 (28.0%)	16 (64.0%)	

All data are expressed as number and percentage. \*Significant difference using  $\chi^2$ , Chi square test at *P* less than 0.05.

control group with no significant difference between Laser and Needle groups.

# Discussion

The present research has revealed that acupuncture could simplify the management of asthma via its antiinflammatory influences. Acupuncture whether laser or needle at specific acupoints has proved to affect Th1/ Th2 balance, block the inflammatory cell activities and immune mediator production, improve bronchial tree remodeling and adjust hypothalamic–pituitary–adrenal axis function. Acupuncture therapy was revealed by prior research efforts to have multiple pathways in managing asthma pathological pathway development [10].

The current study has revealed that reductions in the number of attacks/week and the number of night awakens/month were lowest within the control group without any significant difference concerning Laser and Needle groups. The number of attacks/week and the number of night awakens/month significantly decreased in all the study groups. These research findings are justified by the fact that as long as the respiratory functions are not interrupted during sleep the sleep pattern wouldn't be affected. In addition, it was shown that acupuncture enhances the large, medium and small sized bronchi modeling and reduces bronchial constriction. So, when conjugated with medication, acupuncture leads to augmented improvement in the symptom profile during the disease course. Interestingly, those findings were revealed in a similar fashion in a prior research effort in which it was shown that there is a correlation between bronchial asthma and sleep disorders. Uncontrolled asthma enhances sleep disorders. Also, troubles in sleep, when left without treatment, prevent good management of asthmatic patients [11].

Furthermore, we found that the laboratory changes that reflect the immune system activities, have revealed that one month after treatment, leucocytes, eosinophils, IgE and IL-6 were significantly highest in the control group with no significant difference without any significant differences between the group treated by laser and those treated by needle acupuncture. Hemoglobin levels were not changed significantly in the study groups. Leucocytes, eosinophils, IgE and IL-6 significantly decreased in all the study groups.

Eosinophils cause inflammation when allergens attack the bronchi. Eosinophilia triggering airway inflammation and remodeling leads to persistent bronchial airflow obstruction, epithelial lining damage and increase of the mucus secretion. This is associated with deterioration of the asthmatic manifestations and more impairment of the pulmonary functions [12,13].

A study showed that stimulation of acupoints whether by needles or laser beam can decrease eosinophilia thus, improving the symptoms through regulating disorders in mRNA and promoting eosinophil apoptosis [14]. IgE enhances many immune cells responsible for triggering chronic allergic inflammation through receptors expressed by the airway on the mast cells and eosinophils [15–17]. Also, IgE stimulates the activation and the amplification of the allergens leading to the aggravation of allergic asthma. This occurs mostly after viral infection, such as parainfluenza virus or Epstein-Barr virus. IgE antibodies interacting with the viral infection exacerbate the acute asthma attacks [18].

Acupuncture improves the symptoms of allergic bronchial asthma and the pulmonary function tests by decreasing the serum levels of IgE in children with chronic persistent asthma [19]. Those research findings are justified by the fact that acupuncture has immunomodulatory activities in a manner that reduces the bronchial hyper-responsiveness.

It was revealed, by prior research groups, that either needle or laser acupuncture has proved to be beneficial in decreasing the production of Th2 lymphocytes' cytokines, such as IL4 and IL-5 and increasing the cytokines specific to Th1 lymphocytes leading to the reduction of pro-inflammatory cytokines such as IL1b and IL 6 [20,21]. Also, Park *et al.*, in their trial showed that acupoints stimulation leads to the reduction of the blood levels of IL-4, IL-5 and IgE in an asthmatic mouse [22].

# Conclusion

The presented study concluded that the needle and laser acupuncture proved efficiency in relieving the symptoms of the bronchial asthma in children more than conventional medications alone, as well as Eosinophil, Ig E and IL6 levels were significantly decreased after the treatment.

### Recommendations

Acupuncture is an effective complementary mode that should be integrated in management protocols of asthma in children. However more studies should be performed on larger number of patients to reach more accurate results concerning the anti-inflammatory and immunomodulatory effect of needle and laser acupuncture in asthmatic patients.

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Nil.

# **Conflicts of interest**

There are no conflicts of interest.

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# Evaluation of the efficiency of laser acupuncture in management of chronic lateral epicondylitis in adolescents

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### Background/aim

Lateral epicondylitis known frequently as tennis elbow, is a common disease affecting the elbow joint. It affects 1 to 3% of the population yearly especially individuals in their forties, although the pain has usually a gradual onset, it may occur abruptly. Laser acupuncture is a complementary modality that is used to stimulate the acupoints by low-power laser beam instead of the traditional needles. The present study aims to evaluate the efficiency of laser acupuncture in the management of chronic lateral epicondylitis in adolescents.

### Patient and methods

This study is a randomized controlled trial study on sixty adolescent patients with lateral epicondylitis visiting the acupuncture and laser clinic in excellence Medical Centre, National Research Centre, Cairo, Egypt, during the first of November 2023 till the end of January 2024. The patients were divided into two groups (30 each). The first group was subjected to low-power laser and brufen 400 mg twice daily for 4 weeks, while the patients in the second group were given just the same dose of brufen and used as a control. Pain score, patient rated elbow score, and interleukin-6 (IL-6) were measured before and after the end of treatment. IL-6 was measured by Elisa techniques.

### Results

The present study exhibited insignificant differences (P>0.05) between the studied groups of adolescents with chronic lateral epicondylitis regarding pain score and Patient elbow score before intervention and then it became significantly lower (P<0.05) in both groups group at the end of treatment with Laser and brufen or without Laser. However, adolescent patients subjected to laser and brufen exhibited more reduction (P<0.01) than in the control group treated with brufen only. In addition, the levels of IL-6 exhibited more reduction in the Laser group (P<0.01) than in the control group.

### Conclusion

Low-power laser is efficient in pain reduction and improvement of elbow joint function when 200 mw power, continuous wave, and 12 Joules energy is applied on each acupoint in chronic lateral epicondylitis adolescent patients.

### Keywords:

adolescent, interleukin-6, laser acupuncture, lateral epicondylitis

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# Introduction

Lateral epicondylitis, known frequently as tennis elbow, is a common disease affecting the elbow joint. It affects 1 to 3% of the population yearly especially individuals in their forties [1]. The patients complain of pain and tenderness in the lateral humeral epicondyle where the tendon of the wrist extensor muscle originates. Although the pain has usually a gradual onset, it may occur abruptly [2,3]. If the condition deteriorates, the pain may be propagated to the posterior side of the forearm, It is increased with wrist extension against resistance or its flexion passively, Functional disability may occur such as a decrease in grip strength [4–6].

Lateral epicondylitis is usually caused by extensive use of the muscles located in the back of the forearm such as extensor carpi radialis brevis [7]. It is frequently associated with tennis, racquet, paddle sports and in professions where there is repetitive wrist extension and supination of the forearm. However, it may be idiopathic as many patients have not been exposed to such activities [8,9].

Although the disease is mostly diagnosed clinically, many investigations may be performed such as plain radiography to check for arthritis of the elbow joint or

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electromyography to exclude nerve compression, Magnetic resonance imaging is the most accurate tool to diagnose muscles and tendon injury [10,11]. Oral nonsteroidal anti-inflammatory drugs orcortisoteroid injected locally are used to alleviate the pain but they may have serious side effects [12,13].

On the other hand, Laser acupuncture proved to be effective in treating pain associated with musculoskeletal diseases. Several trials showed that lateral epicondylitis cases were satisfied by the painrelieving effect of acupuncture and the improvement of the mobility of the forearm [14,15]. Laser acupuncture is a complementary modality that is used to stimulate the acupoints by a light beam instead of the traditional needles. Some studies showed good results [6,16], while others did not prove statistical efficiency in management of pain in lateral epicondylitis [17]. The present study aimed to evaluate the efficiency of Laser acupuncture in management of chronic lateral epicondylitis in adolescents.

# Patients and methods Patients

This study is a randomized controlled trial that was conducted in an acupuncture and laser clinic in excellence medical Centre, National Research Centre, Cairo, Egypt, during the start of November 2023 till end of January 2024. Sixty patients with lateral epicondylitis, were diagnosed clinically and collected from tennis academy in public service center in the faculty of physical education at Helwan University.

## Study design

Patients were classified into two groups, each group included 30 patients. The cases of the first group were subjected to low power laser and brufen 400 mg twice daily (Laser group), while the patients in the second group were given just the same dose of brufen and used as control (Control group).

## Inclusion criteria

The inclusion criteria included patients with increased C-reactive protein, ages ranging between 14 and 17 years, chronic cases (lasting more than 3 months), and unilateral elbow affection.

# **Exclusion criteria**

The inclusion criteria included patients with rheumatoid arthritis, physiotherapy, or nonsteroidal anti-inflammatory medications within the previous 3 months.

## **Ethical consideration**

The present study was conducted with the Code of Ethics of the World Medical Association, according to the principles expressed in the Declaration of Helsinki. This study has been approved by the local Ethics Committee of National Research Centre, Cairo, Egypt with approval number 01470123, written informed consent was provided by the parents of each participant before their inclusion in the study.

# Methods

All the cases were evaluated concerning pain VAS during Cozen's test and tennis elbow evaluation before laser intervention.

## P (VAS) assessment (pain score)

P (VAS) is a validated tool for the assessment of the degree of pain in acute or chronic conditions. Scores are identified by making a mark on zero to 10 lines, where 0 means pain-free and 10 indicates the most severe pain [18].

# Cozen's test

Every case was asked to extend the wrist (in a neutral position) against resistance while sitting in a chair with no armrest, shoulder with slight adduction, and elbow flexed at 90°, with the forearm in pronation. Patients were told to do this maneuver, with as much effort they could, twice with an interval period of two minutes between each attempt. The test is considered positive if the patient experienced pain in the lateral epicondyle [19].

## Patient rated elbow evaluation

It is a questionnaire assessing pain sensation and level of disability in the affected elbow joint. The marks are given range from 0 (painless and full function of the joint) to 100 (intolerable pain with marked deterioration of joint function).

# Laser used and acupoints

The present study applied low power laser (200 mws) with continuous wave (830 nm) on the following acupoints (large intestine 10 (LI 10), LI 11, LI 5, LI 6, Gall bladder 34 (GB 34) and Spleen 9 (Sp 9) (Figs. 1–3). Every point is subjected to laser for 1 min giving energy of 12 Joules per point. The sessions were held 3 times a week for 4 weeks. Pain (VAS) and PREE were performed just after the end of the therapy.

## IL-6 measurement

Blood samples were collected from all patients before and after the end of treatment. The blood samples remained in the test tubes for about 30 min and were

### Figure 1



The sites of LI 5 and LI 6 acupuncture points. Red dot LI 6, large intestine 6; yellow dot LI 5, large intestine 5.

### Figure 2



Sites of LI 11, LI 10 and LI 15. Yellow dot LI 11, large intestine 11; red dot LI 10, large intestine 10; yellow dot LI 5, large intestine 5.

then centrifuged at 6,000 rpm for 10 min and the serum was separated and let at -80°C until measurement of interleukin-6 (IL-6) levels. Determination of serum IL-6 was performed by the enzymatic immunoassay method using the kits supplied by DRG International, Inc. (Springfield, NJ 07081, USA), according to the manufacturer's instructions.

### Statistical analysis

The collected was managed and analyzed using IBM SPSS statistics (Statistical Package for Social Sciences) software version 22.0, IBM Corp., Chicago, USA, 2013. Quantitative data was tested for normality using the Shapiro–Wilk test, then described as mean  $\pm$ SE, and then compared using an independent *t*-test. Qualitative data is described as numbers and percentage and then compared using  $\chi^2$  test. The level of significance was taken at *P* value less than or

## Figure 3



Acupoints complementary for pain.

Table 1	Comparison	regarding ag	e and sex of	the two groups	s of adolescent pat	tients with chronic	lateral epicondylitis

Variables	Laser group (group 1)	Control group (group 2)	P value
Age (years)			
Mean±SD	15.8±1.2	15.5±1.1	^0.302
Sex Number (%)			
Male	15 (50.0)	18 (60.0)	<sup>#</sup> 0.436
Female	15 (50.0)	12 (40.0)	

^Independent *t*-test. # Chi square test.

Table 2	Compari	son rega	rding pai	n score (	VAS-10	)
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Variables	Laser group (Group 1)	Control group (Group 2)	P value
Before	8.7±0.9	8.5±0.8	0.376
After	3.2±2.4	5.3±1.4	<0.001*
Change (%)	-63.2	-37.6	
P value	<0.001**	<0.001**	

All data are represented as Mean±SE. \*Significant difference between the two group, using *t*-test at *P* less than 0.05. \*Significant difference before and after treatment in each group, using *t*-test at *P* less than 0.05.

equal to 0.050. The percent of change between two values has been done using the following formula: (Treated – control/control)  $\times$  100.

# Results

The data presented in (Table 1) showed insignificant differences in age (P=0.302) and number of males and females (P=0.436) between the two groups of adolescents patients with chronic lateral epicondylitis subjected to low-power laser and brufen 400 mg twice daily (group 1), or given just same dose of brufen and used as control (group 2).

The data presented in (Table 2) showed insignificant differences between the studied groups regarding pain score before intervention (P=0.376), then it became significantly lower (P<0.05) in the Laser group comparing to the control group after 4 weeks of treatment. However, Pain score exhibited significantly decreased (P < 0.001) in both groups after 4 weeks of treatment. Moreover, pain was significantly more reduced in the Laser group with a percentage of change of -63.2 than control group (-37.6%) that was treated with brufen only.

Regarding the results of patient-rated elbow evaluation score, as shown in (Table 3). It showed insignificant differences between the studied groups regarding elbow evaluation score before intervention (P=0.497), while after 4 weeks of intervention, it became significantly lower (P<0.05) in Laser group than control group. However, the inflammation score exhibited significantly decreased in both groups (P<0.001). In addition, it was reduced in Laser group (-60.5%) than in the control group (-37.1%).

Regarding the results of serum IL-6, it was found that insignificant differences between the levels of serum IL-6 in the two studied groups before intervention (P=0.224), while after 4 week of intervention, it became significantly lower (P<0.05) in Laser group than the control group. However, serum IL-6 exhibited a significant decrease (P<0.001) in both groups after 4 weeks of intervention. Moreover, IL-6 was significantly more reduced in the Laser group (-62.1%) than in the control group (-43.4%), as shown in (Table 4).

### Discussion

In recent years, the interest in the use of laser therapy has increased gradually, although there is still heterogeneity in research data and findings, however, Low-power lasers began in Russia in the early 60's of the twentieth century. Low-power lasers have proved to be effective in the treatment of pain and inflammation through light beams instead of the

 Table 3 Comparison regarding patient rated elbow evaluation score

Variables	Laser group (Total=30)	Control group (Total=30)	P value (groups)
Before	81.7±6.4	82.8±6.5	0.497
After	32.3±17.7	52.1±11.8	<0.001*
Change (%)	-60.5	-37.1	
P value	<0.001**	<0.001**	

All data are represented as Mean±SE. \*Significant difference between the two group, using *t*-test at *P* less than 0.05. \*Significant difference before and after treatment in each group, using *t*-test at *P* less than 0.05.

Variables	Laser group (Total=30)	Control group (Total=30)	P value (groups)
Before	119.9±8.0	121.9±4.6	0.224
After	45.4±25.1	69.0±17.2	<0.001*
Change (%)	-62.1	-43.4	
P value	<0.001**	<0.001**	

Table 4 Comparison regarding serum interleukin-6 (pg/mL)

All data are represented as Mean±SE. Significant difference between the two group, using *t*-test at *P* less than 0.05. Significant difference before and after treatment in each group, using *t*-test at *P* less than 0.05.

heat produced by high-power laser devices [20]. However, the mechanism of action of low-power laser is still controversial. It may enhance the release of serotonin and trigger anti-inflammatory effects [21].

Moreover, low-power laser may increase the intracellular messengers such as adenosine triphosphate and ionized calcium. Also, it can accelerate collagen synthesis which decreases oxidative stress and improves the fibrosis of the tendon. This helps muscle healing and alleviate inflammation and pain [22].

Low-power laser can also relieve the pain through increasing the secretion of morphine like substances such as beta- endorphins and enkephalins. Additionally, it may inhibit pain signals in A-delta and C fibers. Low-power laser increases the blood flow, thus improving blood vessels permeability and boosting the metabolic response. This helps combating inflammation, repair damaged cells, and improve pain [23].

In the current study, pain on visual analog scale and patient rated elbow evaluation showed significant improvement after low-power laser treatment. To our knowledge, this is the first trial accomplished on adolescents. Many studies showed a significant reduction of pain and increased functional elbow capacity in lateral epicondylitis adults treated with low-power laser.

Ercan *et al.* [24] used low-power laser device at a wavelength of 904 nm, a power of 240 MW in a pulsed mode (5,000 Hz of frequency) over the ashi points. Each point was subjected to laser for 30 s. Laser sessions were performed three times per week for 3 weeks. A significant alleviation of pain and restoration of mobility of the joint had been detected in patients (18–64 years age) although the total energy applied to each point (7.2 joules) is less than what used in our study (12 joules). Contrary to the current study, Ercan *et al.* [24] clinical trial did not include a control group.

A meta-analysis study conducted by Bjordal, *et al.* [25] showed significant pain relief in 10 trials including lateral epicondylitis patients treated by lower power laser just after the last laser session. All these trials had control groups that showed also decrease in pain on visual analog scale but less significant than the laser groups.

Lam *et al.* [26] and Stergioulas [27] applied low-power laser (904 nm) directly to the affected tendon. They detected that the pain was significantly reduced more than placebo. Another trial using low-power laser with shorter wavelength (632 nm) than ours (830 nm) showed also significantly better results than ultrasound therapy [28]. However, Bisset and Vicenzino [29] and Basford *et al.* [30] showed no significant pain improvement after laser sessions. This may be due to inadequate laser power or energy.

In the current study, low-power laser proved significant decrease in serum pro-inflammatory IL-6 levels in both group treated with Laser and brufen or with brufen only. Moreover the reduction in cases treated with Laser exhibited more reduction than in cases treated by brufen only and used as control. In agreement with our study, many published papers concluded the anti-inflammatory effect of the low-power laser and its efficiency in lowering the serum levels of IL-6 [31–33].

# **Conclusion and recommendation**

Low-power laser is efficient in pain reduction and improvement of elbow joint function when used with the selected parameters (continuous wave, 830 nm wavelength, and energy of 12 joules) on the acupoints specific for lateral epicondylitis.

We recommend more attention to diagnose lateral epicondylitis in the age group selected in this study which is the first trial performed on adolescents from 14 to 17 years. More number of patients should be included in further studies, follow-up of the cases for 3 to 6 months after termination of laser sessions and higher energy (more than 12 Joules per point) can be applied.

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### **Conflicts of interest**

There are no conflicts of interest.

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# Assessment of BMI in obese patients after semiconductor laser acupuncture therapy

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### Background/aim

There is an urgent need to diminish BMI due to the increased prevalence of chronic critical diseases accompanied by overweight and obesity. Laser acupuncture (LA) is a complementary modality that might lower the BMI by diminishing the abdominal and visceral adipose tissue content and influencing the regulation of the lipid metabolism process. It is a painless tool which is almost free from side effects. The aim of the present study is to assess the BMI in obese patients after semiconductor LA therapy.

### Patients and methods

This is a randomized clinical trial study that enrolled 111 obese patients who visited the Acupuncture and Laser Clinic, Excellence Medical Centre, National Research Centre, Cairo, Egypt. They received 19 successive LA sessions for 2 months without any intervention concerning their routine lifestyle. Low-level laser irradiation was applied at certain acupuncture points according to the principles of Traditional Chinese Medicine. Laser parameters were wavelength 850 nm, and power density 200 mW. Calculated energy 6 J/cm<sup>2</sup> for 24 s per acupoints. The anthropometric measurements were done on all participants before and after the end of LA sessions.

### Results

The present result indicated that there is a significant decrease in weight (P<0.05), with a percent of changes –5.8%. Additionally, the BMI showed a significant decrease (P<0.05), with a percent of changes –6.1% after 2 months of stimulated LA sessions compared to before laser therapy. Also, more than 15 sessions gave 95% sensitivity, 100% specificity for successful significant response, and the number of LA sessions had a significant good utility in predicting successful responses in weight reduction and BMI management.

### Conclusion

The upgrading of the conducted LA sessions had a promising value in different grades of obesity and was safely applicable for BMI management.

### Keywords:

acupuncture, BMI, low-power laser, obesity

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## Introduction

Obesity proved to worsen the prognosis of coronavirus disease 2019 (COVID-19) patients. The increased BMI may aggravate the symptoms of the disease, leading to hospitalization with a higher mortality rate [1]. Consequently, the increased BMI that resulted in overweight and obesity is a great threat to health, economic, and social stability worldwide. Obesity increases the probability of developing the metabolic syndrome (hypertension, hyperlipidemia, diabetes mellitus), and cardiac diseases such as myocardial infarction, heart failure, and malignancy. Any of these diseases are accompanied by an increased death rate in COVID-19 cases [2]. However, the impact of obesity on COVID-19-infected patients is controversial in literature. Many studies showed no correlation between obesity and deterioration of COVID-19 cases [3].

The BMI is usually performed to obtain the outcome of the nutritional status of the patients. BMI is a simple way to identify overweight and obesity. It is calculated by dividing the weight in kilograms by the square and the height in meters. Patients having a BMI higher than 25 and less than 30 are considered overweight. BMI, which is more than 30, indicates obesity, which is classified into three classes (first from 30 to 35, second from 35 to 40, and third above 40) [4]. Obesity leads to the death of four million people worldwide because of its cardiovascular complications. Five percent of children and 12% of adults have obesity globally [5].

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Obesity is caused by hormonal disturbances, sedentary life, and foods high in calorie. The increased consumption of saturated fats from animal sources, white bread, white flour, high-sugar beverages, and processed foods causes obesity. A balanced diet regime and a physically active pattern of life are essential for controlling obesity. Medications and even surgery may be needed in some cases [6].

The American Food and Drug Administration approved five medications for obesity management. However, orlistat is the only one available globally [7]. Also, all these medications fail to decrease body weight by more than 5% in most cases [8]. They are frequently associated with many side effects, such as diarrhea, vertigo, headache, nausea, vomiting, inverted sleep rhythm, hypertension, and even cardiovascular diseases [7,9]. New antiobesity medications are under trials and seem to be promising, but not yet approved [9].

Recently, laser acupuncture (LA) treatment of obesity showed good results but lacked comparative studies due to different laser parameters applied [10]. LA is a technique used to stimulate acupoints with lowpower laser light instead of the traditional needles. Laser is painless and safe and preferred over needles by many patients [11]. LA action is transmitted via the afferent fibers to the central nervous system, then, in turn, activates both the hypothalamus and limbic system. Therefore, the appetite center in the hypothalamus could be regulated [12].

LA efficiency is not restricted to obesity treatment but may also manage symptoms accompanied by obesity, such as constipation, heavy body sensation, and menstrual cycle disorders. During laser sessions, patients are educated concerning diet regimes, suitable exercise, and healthy patterns of life. LA exerts its therapeutic effect through actions on various biochemical markers such as leptin and ghrelin. Laser also adjusts lipid, glucose metabolism, insulin sensitivity, and inflammatory markers [13]. Therefore, the benefits of LA may be better than the weight loss diet regime [14]. However, evaluating the beneficial effect of LA is still challenging due to the deficiency of high-quality clinical trials with sufficient treatment sessions, so the aim of the present study is to assess the BMI in obese patients after semiconductor LA therapy.

# Patients and methods Patients

This interventional randomized clinical trial study was conducted at Acupuncture and Laser Outpatient Clinic, Medical and Scientific Center of Excellence, National Research Centre, Cairo, Egypt. In this clinical trial, we recruited 111 patients, ages range from 15–18, with calculated BMI more than 25.0. The exclusion criteria included patients who received anti-inflammatory steroid therapy and medications for hypercholesterolemia, diabetic patients, patients with chronic digestive disorders more than 3 months (diarrhea, constipation, and gastritis), abnormal kidney or liver functions, and pregnancy were also excluded from the study. Finally, cases who performed regular exercise at least two to three times per week (30–45 min each session) or who had contraindications to LA were also excluded.

# Study design

Patients had received successive laser acupuncture sessions over the 2 months study period, with a frequency of two sessions per week. The anthropometric measurements were done on all patients precompletion and postcompletion of the LA sessions.

# Ethical considerations

This study was considering the ethical rules for the medical research involving human participants according to the Declaration of Helsinki in 1964 and was reviewed and approved by the Ethical Committee of the National Research Centre, Cairo, Egypt, with approval number 01431223. All participants signed a written illustrative and informed consent prior to their inclusion in the study.

# Anthropometric measurements

Body weight and height were measured, following the recommendations of the 'International Biological Program' [6]. Body weight was determined to the nearest 0.01 kg using a Seca Scale Balance, with the participant wearing minimal clothes and no shoes. Body height was measured to the nearest 0.1 cm using a Holtain portable stadiometer. BMI=weight (in kg) divided by height (m<sup>2</sup>), was calculated. Four categories of BMI of the study population were tabulated in Table 2 according to the WHO issued guidance about BMI [4].

# Acupuncture points

The stimulated acupoints were used according to the principles of Traditional Chinese Medicine and the recommendations of evidence-based studies of the management of obesity and overweight with acupuncture [15]. The body acupoints used were ST25 (Tianshu), SP15 (Daheng), bilateral Quchi (LI11), bilateral ST36 (Zusanli), bilateral Sanyinjiao

(SP6), Guanyuan (CV4), and Zhongwan (CV12). In addition to the Shen Men (TF4) auricular acupuncture point that was applied with routine ear-pressing plasters (Vaccaria ear seeds; Beijing Zhongyan Taihe Medicine, Beijing, China). After sterilizing the acupoints with 75% alcohol preparation pads, the acupuncturist inserted the ear-pressing plaster with the seed into the acupoints on a single ear alternatively during the two sessions per week thus to be kept on the ear for 3 days. All participants were requested to apply pressure to the auricular points before eating.

### Laser acupuncture session

LA was directed to each body acupoint continuous wave's 200 mW power density, and 6 J/cm<sup>2</sup> for 24 s per acupoint with wavelength 850 nm. The participating group had received direct contact low-level LA therapy for a maximum 19 sessions with a frequency of two sessions per week. Laser-protection goggles were asked to be worn during each session. There was no reported adverse effect due to laser irradiations.

### Statistical analysis

Data were gathered, authenticated, encoded, and analyzed using the Statistical Package for the Social Sciences (SPSS), version 22. (SSPS Inc., Philadelphia, Pennsylvania, USA). The mean and SD were presented for continuous variables. The independent t test was used to compare quantitative data between the two groups. While qualitative data is described as numbers and percentages and then compared using  $\chi^2$ test. Pearson's correlation analysis was used when looking at the relation between two quantitative variables within the same group. The variables that had the greatest effect on inflammatory mediators between cases were identified using multiple linear regressions. The level of statistical significance for all tests was set at P value less than 0.05. The percent of

Table 1 Demo	ographic	characteristics	of	obese	cases
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Variables	
Age (year)	16.3±0.7
Sex	
Male	23 (20.72)
Female	88 (79.28)
Height (m)	1.628±0.073

Data expressed as mean $\pm$ SD and *n* (%).

change between two values has been done using the formula: (treated-control/control)×100.

### Results

The present study enrolled 111 obese participants in age range of 15–18 years old. Demographic data is shown in Table 1. The majority of the study populations were female 79.28%, while the male were 20.72%, with mean height  $1.628\pm0.073$  m, weight  $99.49\pm22.67$  kg, and BMI  $37.50\pm7.80$  kg/m<sup>2</sup>. More than 1/3 of cases had morbid obesity and half of cases with class I (*n*=33) and class II (*n*=23) obesity, while cases that were enrolled in class III were 38 cases.

The anthropometric measurements in obese patients before and after 2 months of LA sessions were reported in Table 2. The weight of obese cases after LA therapy exhibited significant decreases (P<0.05) than the weight of cases before laser therapy (93.69+22.03 vs. 99.49+22.67 kg/m<sup>2</sup>), with a percent of change -5.8% reduction. Additionally, the BMI showed significant decreases (P<0.05) than the BMI of obese cases before therapy (baseline) (35.23+7.33 vs. 37.50+7.80 kg/m<sup>2</sup>), with a percent of change -6.1% reduction. Moreover, the frequency of obesity classes showed significant changes (P<0.05) after the completion of the 12 successive LA sessions. Furthermore, more than 1/3 (37.84%) of cases responded well during the study duration, as shown in Table 2.

Variables	Before laser therapy (baseline)	After laser therapy
Weight (kg)	99.49±22.67	93.69±22.03*
Change difference (%)		-5.8
BMI (kg/m <sup>2</sup> ) <sup>#</sup>	37.50±7.80	35.23±7.33*
Change difference (%)		-6.1
Obesity classes**		
Overweight	17 (15.32)	26 (23.42)
Obesity class I	33 (29.73)	32 (28.83)
Obesity class II	23 (20.72)	29 (26.13)
Obesity class III	38 (34.23)	24 (21.62)
Weight reduction response		42 (37.84)

Table 2 BMI in obese patients before and after 2 months of laser acupuncture therapy

Data expressed as mean±SD and n (%). Significant difference than before treatment at P value less than 0.05, using t test. Significant difference than before treatment at P value less than 0.05, using  $\chi^2$  test.

Figure 1



Pearson's correlation between percentage of changes differences in weight (a) and BMI (b) after two months of therapy with the number of laser acupuncture sessions.

Table 3 Utility of laser session number in perdition of successful weight reduction

Cutoff	Sensitivity (%)	95% CI	Specificity (%)	95% CI	PPV (%)	NPV (%)
11.5	64	0.4803–0.7845	67	0.5429-0.7756	30	89
15.5	95	0.005820-0.1616	100	0.9479-1.000	100	83

PPV, positive predictive value; NPV, negative predictive value.

The minimum number of recorded LA sessions in the study duration was three successive sessions, while the maximum number was 19 sessions. Successive upgrades in laser stimulation of acupuncture points led to remarkable cumulative effects, resulting in significant control of BMI. However, Pearson's correlation between the change difference percent in weight and BMI were significantly positive with the number of LA sessions (r=0.190, P=0.030 and r=0.220, P=0.010, respectively), shown in Fig. 1.

At cut-off point more than 11 sessions, the sensitivity and specificity 64 and 67%, respectively. However, more than 15 sessions gave 95% sensitivity, 100% specificity, and positive predictive value for a successful significant response (Table 3). The number of LA sessions had a significant utility in predicting successful response in weight reduction, the area under the curve=0.670, P=0.003 (Fig. 2).

# Discussion

This interventional study enrolled 111 overweight and obese patients. They were subjected to laser body acupuncture. A significant reduction in body weight and BMI had been detected. The mechanism of action of LA in the management of obesity was controversial. However, many studies showed that it has the same mode of action as needle acupuncture. All participants signed a written illustrative and informed consent prior to the study. All participants signed a written illustrative and informed consent before the study [16,17].





ROC curve of laser session number in predicting successful weight reduction response. ROC, receiver operating characteristic.

MRI has demonstrated that LA stimulates centers in the brain that boost the body's metabolism [18]. Von Deneen *et al.* [19], proved that acupuncture affects many neurophysiological pathways, such as dopaminergic signaling. Acupuncture also increases the secretion of satiety hormones and the basal metabolic rate.

Furthermore, LA was reported to induce the formation of several temporary pores in the adipocytes enhancing apoptosis leading to release of lipids [20]. LA can also affect the gene expression in the adipocytes inducing changes in their morphology [21,22]. These changes stimulate the mitochondria to increase cyclicadenosine monophosphate and adenosine triphosphate synthesis. Thus, lipase enzyme is activated and triglycerides are broken and converted into fatty acids and glycerol [23].

Consecutively, LA might be suggested to suppress the production of ghrelin hormone, leading to a decrease hunger sensation [24]. Additionally, it can reduce blood glucose and insulin resistance [24,26]. Thus, leading to increase of insulin sensitivity resulted in reduction of several inflammatory cytokines such as interleukin 1, interleukin 8 and tumor necrosis factor. Meanwhile, the improvement of insulin sensitivity facilitates weight reduction [27–29].

Agouti gene-related protein is a peptide hormone expressed in the arcuate nucleus of the hypothalamus. Its release was increased during fasting, inducing hunger and leading to obesity. Stimulating acupuncture points ST36 and LI11 decreases both appetite and body weight through the inhibition of agouti gene-related protein expression in obese rats [30].

Since, cholecystokinin is secreted from the gastrointestinal system and transmitted to the brain through the vagus nerve. Therefore, activation of cholecystokinin had resulted in decreased appetite and weight reduction [31], which could be achieved by applying acupuncture sessions to ST40, CV12, and CV4 [32]. Moreover, stimulating the abdominal acupoints (ST25, CV4, and CV12) and lower limbs acupoints (ST36 and ST40) was observed to decrease weight by increasing serum cholecystokinin and decreasing leptin [33].

Concerning auricular needle acupuncture, it was found that stimulating specific ear acupoints may induce action potentials in the hypothalamic ventromedial nucleus of the hypothalamus which inhibit hunger sensation. Auricular acupuncture might decrease neuropeptide Y which increases the desire to eat and delays satiety. Keeping on, it increases the basal metabolic rate by enhancing a sympathomimetic effect. It helps in weight reduction through increasing insulin sensitivity and combating inflammatory cytokines same as laser body acupuncture [34].

Low-power laser has been approved by the Food and Drug Administration as a safe, painless, noninvasive modality to treat simple generalized and localized obesity [35]. However, there is marked variations of power, energy and frequency of laser sessions applied in the literature. In many research papers, laser parameters used are not mentioned. We used GaAlAs laser with 850 wavelength, continuous wave, 200 mW power and 6J were applied on each acupoint with frequency two times per week. Sebayang et al. [36] used lower power (50m W) than we applied plus lower energy over each point (4]). However, they did not mention the wavelength of their laser device. They concluded that LA is statistically more effective in reducing weight when combined with diet regime than diet intervention alone. We tried higher joule in our study because world association of laser therapy recommended that lowpower laser is safe whatever the energy applied as long as we exclude the cases where laser should be contraindicated [37].

Woźniak *et al.* [38] proved also that combining LA with a low calorie diet was more effective in decreasing body weight, BMI, and waist hip ratio than low caloric diet alone in menopausal women. On the contrary, El-Mekawy *et al.* [16] and Hassan *et al.* [39] showed no improvement statistically in group of patients receiving LA and calorie-restricted diet over those on diet only concerning weight, BMI, and waist hip ratio. This may be due to very low caloric diet given to all the patients included in the study who were classified as class II obesity.

All the previous studies including ours, did not have a control group where cases are exposed to sham laser. In the literature, many trials had been performed including control groups. Tseng *et al.* [17] showed that LA decreased body weight, BMI, waist circumference, waist hip ratio when compared with sham laser (control group). However, no two groups Tseng *et al.* [17], also recorded that LA suppress the appetite by increasing fullness sensation and decreasing the feeling of hunger.

Hung *et al.* [25] concluded that BMI and free fat mass decreased in the group receiving low-level LA therapy compared with the control group exposed to sham laser after about 3 weeks of treatment. Laser was given five sessions per week (total of 15 sessions). In our study, BMI showed significant decrease after the same period of time. However, we gave two sessions per week and improvement was detected after session 11 in the third week. Reduction of weight was more significant when period of time was extended. Patients given 19 sessions (over 6 weeks) showed better results.

On the contrary, Liu *et al.* [40] showed that LA showed no improvement over sham laser. They applied 24 LA sessions with no significant body weight and BMI improvement over the sham group. This negative result may be due to stimulation of only two acupoints (ST25 and SP15). We conducted the laser device over 11 acupoints according to the traditional Chinese medicine guidelines [41].

In literature, many published articles demonstrated the efficiency of auricular acupuncture in management of overweight and obese patients, whether used alone or combined with laser body acupuncture as in this trial. A systemic review and meta-analysis of randomized controlled trials recorded a significant reduction in weight, BMI, body fat and waist circumference. All these parameters showed a 95% confidence interval [42–46].

### Conclusion

In conclusion the cumulative influence of the successive conducted LA sessions showed a promising value in reducing body weight and BMI. However, further studies with different low-level LA parameters should be performed.

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### **Conflicts of interest**

There are no conflicts of interest.

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# Assessment of the changes in nutritional and psychological behaviors of Saudi adults related to the impact of the coronavirus infectious disease-19 curfew: A cross-sectional study Inas R. El-Alameey<sup>a,b</sup>, Ghadi A. Aljohani<sup>a</sup>, Rawan R. Alharbi<sup>a</sup>, Rana A. Shuwaykan<sup>a</sup>, Amal E. Almutairi<sup>a</sup>, Aram A. Shokr<sup>a</sup>

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### Background/aim

In response to the coronavirus infectious disease - 19 (COVID-19) spread, the Saudi government enforced a nationwide curfew, which in turn has influenced the dietary habits and psychological behaviors of the Saudi population. Data regarding the impact of curfew on Saudi adults are lacking. Thus, the study aims to assess the changes in nutritional and psychological behaviors of Saudi adults related to the impact of the coronavirus disease-19 curfew.

### Patients and methods

This study is a cross-sectional online study among Saudi adults aged between 25 and 65. This study includes 1040 adults from different regions of Saudi Arabia. Data regarding nutritional behaviors, physical activity, weight changes, and psychological behaviors were collected via an online questionnaire. The psychological behaviors during the curfew were assessed by Depression, Anxiety, and Stress Scale-21. The Chi-square test was used to test for the presence of an association between the different categorical variables.

### Results

Out of 1040 participants, 76.9% were female, and 36.4% were aged between 46 and 65 years. Our results showed an increase in intake in dessert by 54.2%, salty snacks by 52.1%, and appetite by 48.9%. Over 24.7% and 45% of participants reported eating more meals and snacking, respectively. Half of the participants gained weight, especially among male participants (P=0.012). Physical activity had also decreased among male participants (P<0.001). The prevalence of moderate to extremely severe anxiety, stress, and depression during the curfew was noted to be 13.8, 3.7, and 8.4%, respectively, without any gender difference.

### Conclusion

Public health officials should focus on increasing awareness of healthy eating habits and provide psychological support and emphasizing physical activities during the curfew.

### Keywords:

adults, coronavirus disease-19, curfew, nutritional behaviors, psychological behaviors

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## Introduction

The coronavirus disease-19 (COVID-19) was first reported at the end of December 2019, with only a few cases in Wuhan, China [1]. Since COVID-19 has spread rapidly across the world within a relatively short time, the World Health Organization (WHO) declared COVID-19 as a global pandemic in March 2020 [2]. As a result of the outbreak, Saudi Arabia's government made massive efforts to prevent COVID-19 from spreading. The government enforced a curfew for all its residents, imposed social distancing rules, and banned attendance at workplaces, schools, restaurants, shops, and gyms [3].

While these precautions are essential for slowing the virus's spread, they may adversely affect people's daily routines and lifestyles [4]. This study focuses on adults

as they are considered the foundation of the family as well as playing a critical influence on shaping their children's nutritional and psychological behaviors. Despite the limited data on the impact of the COVID-19 curfew on adults' lives, previous studies have shown that the COVID-19 curfew has a negative impact on people's dietary habits, lifestyles, and psychological behaviors [5,6].

The curfew period has resulted in changes in people's meal patterns, including quantity, frequency, and food quality. Several studies in Poland, France, and the

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United States of America [7–9] have assessed people's food intake during the COVID-19 curfew. The findings revealed negative dietary trends such as increased caloric consumption, more frequent snacking, and a reduction in fresh fruit and vegetable consumption. Furthermore, the negative dietary habits during the COVID-19 curfew were consistent with a study conducted in Saudi Arabia, which found that over 40% and 45% of participants reported overeating and oversnacking, respectively [5]. The females were more inclined to increase in food amounts during the curfew according to a study conducted in Bavarian [10].

Information related to the impact of the COVID-19 curfew on food quality in Saudi Arabia is limited. However, in general, there was an increase in the consumption of sweets, including chocolate, cakes, and ice cream [5]. Similar findings in the United Arab Emirates show that sweet and salty snacks were reported among food items whose intake increased greatly during the curfew [11]. In addition, people in a negative mood may tend to eat energy-dense, palatable foods that are high in sugar content, which positively impacts mood levels. Therefore, the concerned authorities should increase nutrition awareness by suggesting healthy and nutritious food choices to limit the adoption of unhealthy dietary habits during pandemics, especially in curfew situations.

Since the curfew limits people from going to work, fitness centers, and even performing regular daily activities, several studies have shown that the COVID-19 curfew had an adverse impact on people's physical activity (PA) [5]. A study in Saudi Arabia examined the effect of the COVID-19 curfew on PA. The results indicated that 80.6% of participants were not meeting the WHO recommendations regarding PA during the curfew [12]. Additionally, sex differences in PA were observed in the United Arab Emirates, as they declared that 50% of male participants showed a reduction in their PA level during the curfew [13].

As a result of the prevalence of sedentary lifestyles and unhealthy dietary habits, weight gain during the curfew was anticipated. Several studies around the world examined the weight change during the curfew. In Saudi Arabia, a study reported weight gain of around 3–5 kg in 38% of the participants during the curfew, while 26% and 34% reported a decrease in weight and no change, respectively [5]. Besides, a study conducted in the United Arab Emirates showed that 36.1% of male participants declared an increase in weight during the COVID-19 curfew. Consequently, more support and attention should be paid to obese and overweight individuals related to the positive effect of losing weight on health during the curfew.

The curfew during disease outbreaks raised many psychological issues, including loss of freedom, uncertainty over disease status, separation from loved ones, and boredom, which occasionally had pernicious effects. Various studies reported developing general psychological symptoms, traumatic stress, depression, insomnia, emotional disturbance, anger, and irritability in the adult population during the curfew [9,14].

A cross-sectional study among the public of Saudi Arabia declared that 23.6% of the participants showed moderate or severe psychological impact of the curfew. Additionally, 22.3, 24, and 28.3% of participants reported moderate-to-severe stress, anxiety, and depressive symptoms, respectively. The increased psychological impact triggered by the enforced curfew should encourage policymakers to implement public mental health strategies designed to mitigate the adverse effects associated with the curfew [15]. Thus, this study aims to assess the changes in the nutritional and psychological behaviors of adults related to the impact of the COVID-19 curfew in Saudi Arabia. Moreover, to investigate how adults' eating habits, PA, weight changes, and psychological behavior have been affected during the curfew in Saudi Arabia and the relationship between these changes with respect to sex.

# Patients and methods Participants and recruitment

A sample of 1040 adults from different regions of Saudi Arabia was recruited. The inclusion criteria were citizens and residents of Saudi adults aged between 25 and 65 years. Participants were requested to fill out an electronic questionnaire that was created via Google Forms and distributed on various platforms, such as WhatsApp, Twitter, and Snapchat groups. To reach all adults in society, data were collected using random convenience sampling and the snowball technique.

# Ethical approval

Ethical approval for this study was obtained from the Ethical Committee of the College of Applied Medical Sciences at Taibah University, Al-Madinah Al-Munawara, Saudi Arabia, with approval number 2020/59/206 CLN. All participants provided informed consent through a statement on the questionnaire to be included in the study.

### Sample size calculation

The representative target sample size needed to achieve 40% of expected changes in dietary habits, a confidence level of 95%, and a 3% margin of error, was calculated by Epi Info TM [16].

## The questionnaire

This questionnaire was adapted from this study [5], however, some of the questions were modified to be answered sequentially and in accordance with the scenarios 'before' and 'during' the curfew. The selfreported questionnaire was translated from English to Arabic by the authors to be distributed. The questionnaire's reliability was tested through piloting before survey administration for clarity and logical flow of the questions.

On the first page of the online questionnaire, a proper explanation about the study objectives, the estimated time of completion, and the confidentiality of collected data has been made to the respondents. All participants provided informed consent through a statement on the questionnaire in order to be included in the study. The survey needed around 10–15 min and was made available online from October 15, 2020 to February 15, 2021.

This questionnaire comprises four main sections with 20 questions in total. The first section gathered sociodemographic information on respondents' characteristics, including age, sex, educational level, employment status, region of residence, family income, and whether there have been income changes during the pandemic or not. The second section assessed the change in the participants' dietary habits and nutritional intake during the curfew as compared with before. This section included 9 items on modes of the number of meals and snacks, change in portion size, food groups' intake, appetite, weight status, supplement use and consumption of immune-boosting food, and participation in cooking. The main food groups adapted from the Saudi Dietary Guideline 'The Healthy Food Palm' include cereal and bread, meat and eggs, milk and dairy products, vegetables, and fruits [17]. Additional food groups were added to categorize snacks and drinks such as desserts, salty snacks, juices and soft drinks, coffee, and tea.

The third section assessed the change in PA level using three questions: (1) change in PA during the curfew as compared with before, (2) average time spent actively during the week, and (3) average daily screen time spent during the curfew and before. To describe the change, the respondents chose one of three categories: 'decreased,' 'increased,' or 'it has not changed.'

The fourth section assessed the participants' mental health status during the curfew as compared with before by using an Arabic version of the Depression, Anxiety, and Stress Scale-21 (DASS-21) [18]. DASS-21 is a self-report questionnaire consisting of 21 items concerning three subscales: depression, anxiety, and stress. Each subscale consists of seven items with four option scales ranging from 0 (did not apply to me at all) to 3 (applied to me very much or most of the time). The final scores of each subscale were compared with DASS severity ratings, which were categorized into normal, mild, moderate, severe, and extremely severe.

### Statistical analysis

Statistical analysis was performed using Statistical Packages for Software Sciences (SPSS) version 21, Armonk, New York, IBM Corporation. The normality of the data distribution was confirmed using the Shapiro-Wilk test as well as the Kolmogorov–Smirnov Frequency test. and percentages were used to display categorical variables. A Chi-square test was conducted to test for the presence of an association between categorical variables. Statistical significance was identified at P less than 0.05.

## **Results**

About 1040 adults were enrolled to measure the effect of the COVID-19 pandemic on the nutritional and psychological behavior of adults before and during the curfew in Saudi Arabia. Table 1 presents the sociodemographic characteristics of 1040 adults. The most common age group was 46-65 years old 36.4%, the majority were female 76.9%, and most were university degree holders 61%. With respect to their monthly income, more than half 52% were earning above 10 000 SAR per month and 25.1% were earning 6,000–10 000 SAR/month. Furthermore, nearly half of them, 45.3%, were working in the government sector, while 30.4% were unemployed. With regard to the impact of the current pandemic on respondents' family income levels, approximately half of them, 49.8%, stated that their income did not change, while 38.6% stated it decreased and only 11.6% stated it increased. In addition, most of the respondents, 69.8%, were living in the Western region.

In Fig. 1, it can be noted that eating three meals per day was higher before the curfew 62.2% than during the

Table 1	Sociodemographic	characteristics	of all adults'	participants
	Coologiapino	01101001001000		participarito

Study data	Overall [n (%)] (N=1040)	Male [n (%)] (N=240)	Female [n (%)] (N=800)
Age group in years			
25–35 years	295 (28.4)	50 (20.8)	245 (30.6)
36–45 years	366 (35.2)	80 (33.3)	286 (35.8)
46–65 years	379 (36.4)	110 (45.8)	269 (33.6)
Educational level			
Below high school	61 (5.9)	3 (1.3)	58 (7.3)
High school/diploma	249 (23.9)	88 (36.7)	161 (20.1)
University	634 (61.0)	106 (44.1)	528 (66.0)
Master/PhD degree	96 (9.2)	43 (17.9)	53 (6.6)
Monthly income (SAR)			
<4,000	93 (8.9)	11 (4.6)	82 (10.3)
4,000-6,000	145 (13.9)	22 (9.2)	123 (15.4)
6,000–10,000	261 (25.1)	47 (19.6)	214 (26.8)
>10,000	541 (52.0)	160 (66.7)	381 (47.6)
Occupation			
Governmental sector	471 (45.3)	151 (62.9)	320 (40.0)
Private sector	71 (6.8)	29 (12.1)	42 (5.3)
Freelance	29 (2.8)	07 (2.9)	22 (2.8)
Retired	153 (14.7)	44 (18.3)	109 (13.6)
Unemployed	316 (30.4)	09 (3.8)	307 (38.4)
Impact of family income			
Increased	121 (11.6)	34 (14.2)	87 (10.9)
Decreased	401 (38.6)	94 (39.2)	307 (38.4)
No change	518 (49.8)	112 (46.7)	406 (50.7)
Area of residency			
Northern	106 (10.2)	29 (12.1)	77 (9.6)
Southern	37 (3.6)	11 (4.6)	26 (3.3)
Central	122 (11.7)	26 (10.8)	96 (12.0)
Eastern	49 (4.7)	11 (4.6)	38 (4.8)
Western	726 (69.8)	163 (67.9)	563 (70.4)

All data are represented as number and percentage.

curfew 52.4%, while 24.7% of the participants reported eating more than three meals.

In Fig. 2, it can be noted that having one snack and two snacks were higher before the curfew at 42% and 39.1%, respectively, than during the curfew at 21.9%

and 33.2%, respectively, whereas, having more than two snacks was higher during the curfew with 45% than before the curfew with 18.8%.

The following results in Table 2 showed the top nutritional factors where adults increased their



Figure 1

Number of meals before and during coronavirus disease-19 curfew.

Figure 2



behaviors were cooking participation 65%, drinking coffee 57.3%, eating salty snacks 52.1%, eating dessert 54.2%, and appetite 48.9%, while a decrease in the nutritional habit mainly was noted in eating fast food 37.4%, followed by drinking juices and soft drinks 22.4% and eating legumes 20.5%, respectively.

In Table 3, a significant difference between males and females was observed in the change of the following nutritional behaviors during the COVID-19 curfew as compared with before the curfew: grain (P=0.008), meat (P=0.010), tea (P=0.006), weight changes (P=0.012), and cooking participation (P=0.002).

In Table 4, it was observed that 43.9% of the respondents stated that their PA increased during the curfew, while 29.7% showed a reduction in their PA. When measuring the PA in accordance with gender, it was found that change in PA was significantly associated with gender (P<0.001). Male participants reported a decrease in their PA by 44.2%, while female participants reported a decrease in their PA by 25.4%. It was also observed that 55.6% of the respondents reported an increase in their weight as well. Weight changes were significantly associated with gender (P= 0.012). Male participants reported an increase in their weight sociated with gender (P= 0.012). Male participants reported an increase in their weight by 63.8%, while female

Table 2 Change in parents' nutritional behavior during the COVID-19 curfew compared to before the COVID-19 curfew (n=1040)

Variables	Increased N (%)	Decreased N (%)	No changed N (%)	I don't eat N (%)
Cooking participation	676 (65.0%)	55 (5.3%)	309 (29.7%)	_
Coffee	596 (57.3%)	67 (6.4%)	341 (32.8%)	36 (3.5%)
Dessert	564 (54.2%)	174 (16.7%)	266 (25.6%)	36 (3.5%)
Salty snacks	542 (52.1%)	156 (15.0%)	308 (29.6%)	34 (3.3%)
Appetite	509 (48.9%)	138 (13.3%)	393 (37.8%)	-
Water	504 (48.5%)	88 (8.5%)	432 (41.5%)	16 (1.5%)
Теа	493 (47.4%)	86 (8.3%)	416 (40.0%)	45 (4.3%)
Grain	491 (47.2%)	109 (10.5%)	430 (41.3%)	10 (1.0%)
Vegetables	372 (35.8%)	158 (15.2%)	490 (47.1%)	20 (1.9%)
Meal portion	360 (34.6%)	117 (11.3%)	563 (54.1%)	-
Fruit	360 (34.6%)	202 (19.4%)	446 (42.9%)	32 (3.1%)
Meat	340 (32.7%)	166 (16.0%)	522 (50.2%)	12 (1.2%)
Milk products	294 (28.3%)	148 (14.2%)	563 (54.1%)	35 (3.4%)
Juices and soft drinks	277 (26.6%)	233 (22.4%)	352 (33.8%)	178 (17.1%)
Fats	244 (23.5%)	185 (17.8%)	575 (55.3%)	36 (3.5%)
Fast food	244 (23.5%)	389 (37.4%)	240 (23.1%)	167 (16.1%)
Legumes	201 (19.3%)	213 (20.5%)	570 (54.8%)	56 (5.4%)

Variables	Increased [n (%)]	Male decreased [n (%)]	No change [ <i>n</i> (%)]	Increased [n (%)]	Female decreased [n (%)]	No change [ <i>n</i> (%)]	P value
Meal portion	95 (39.6)	30 (12.5)	115 (47.9)	265 (33.1)	87 (10.9)	448 (56.0)	0.087
Grain	133 (56.4)	23 (9.7)	80 (33.9)	358 (45.1)	86 (10.8)	350 (44.1)	0.008 **
Milk products	75 (32.3)	37 (15.9)	120 (51.7)	219 (28.3)	111 (14.4)	443 (57.3)	0.321
Meat	98 (40.8)	38 (15.8)	104 (43.3)	242 (30.7)	128 (16.2)	418 (53.0)	0.010 **
Legumes	45 (19.5)	62 (26.8)	124 (53.7)	156 (20.7)	151 (20.1)	446 (59.2)	0.089
Fruit	91 (38.6)	56 (23.7)	89 (37.7)	269 (34.8)	146 (18.9)	357 (46.2)	0.056
Vegetables	86 (36.6)	42 (17.9)	107 (45.5)	286 (36.4)	116 (14.8)	383 (48.8)	0.468
Fats	67 (28.8)	46 (19.7)	120 (51.5)	177 (23.0)	139 (18.0)	455 (59.0)	0.104
Dessert	125 (54.6)	38 (16.6)	66 (28.8)	439 (56.6)	136 (17.5)	200 (25.8)	0.659
Salty snacks	123 (53.0)	37 (15.9)	72 (31.0)	419 (54.1)	119 (15.4)	236 (30.5)	0.953
Fast food	57 (27.1)	96 (45.7)	57 (27.1)	187 (28.2)	293 (44.2)	183 (27.6)	0.923
Теа	134 (57.8)	22 (9.5)	76 (32.8)	359 (47.1)	64 (8.4)	340 (44.6)	0.006 **
Coffee	138 (59.7)	15 (6.5)	78 (33.8)	458 (59.2)	52 (6.7)	263 (34.0)	0.987
Juices and soft drinks	61 (30.2)	65 (32.2)	76 (37.6)	216 (32.7)	168 (25.5)	276 (41.8)	0.169
Water	128 (54.2)	20 (8.5)	88 (37.3)	376 (47.7)	68 (8.6)	344 (43.7)	0.190
Appetite	113 (47.1)	33 (13.8)	94 (39.2)	396 (49.5)	105 (13.1)	299 (37.4)	0.806
Weight changes	153 (63.8)	25 (10.4)	62 (25.8)	425 (53.1)	122 (15.3)	253 (31.6)	0.012 **
Cooking participation	140 (58.3)	08 (3.3)	92 (38.3)	536 (67.0	47 (5.9)	217 (27.1)	0.002 **

Table 3 Gender difference of change in nutritional behavior during the COVID-19 curfew compared to before the COVID-19 curfew (n=1040)

All data are represented as number and percentage. *P* value has been calculated using  $\chi^2$ , Chi-square test. <sup>\*\*</sup> Significant at *P* less than 0.05 level, using  $\chi^2$ , Chi-square test.

participants reported an increase in their weight by 53.1%.

Figure 3 depicts the PA before and during the COVID-19 curfew. It can be noticed that PA for less than 6 h/week was higher before the curfew 30.7% than during the curfew 11.1%. On the other hand, physical inactivity was higher during the curfew 22.1% as compared with before the curfew 0.8%.

In Fig. 4, it was observed that the most-taken supplement during the curfew was vitamin C 43.8%, followed by vitamin D 24.8%, and vitamin B 13.8%, while folic acid was the least 0.7%.

In Fig. 5, it was observed that the most-taken food items that boosted the immune system were honey 59.2%, followed by citrus 53.9% and ginger 46.8%, while onion was the least taken 35.8%.

The prevalence of anxiety, depression, and stress has been described in Table 5. Based on the results, the prevalence of anxiety was mild, moderate, severe, and extremely severe among the participants with 5.9, 9.6, 2.9, and 1.3%, respectively. With regard to depression, the prevalence of depression was found to be mild, moderate, and severe among 9.2, 6.8, and 1.6% of the participants, respectively. Finally, the prevalence of stress was mild and moderate among 5.7% and 3.7%

Table 4	Physical a	activity and	weight change	during	COVID-19 curfew	in relation to	gender (n=1040)
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Variables	Overall [n (%)] (N=1040)	Male [n (%)] (N=240)	Female [n (%)] (N=800)	P value		
Physical activity char	nge					
Increased	457 (43.9)	82 (34.2)	375 (46.9)	<0.001**		
Decreased	309 (29.7)	106 (44.2)	203 (25.4)			
No change	274 (26.3)	52 (21.7)	222 (27.8)			
Weight change				0.012**		
Increased	578 (55.6)	153 (63.8)	425 (53.1)			
Decreased	147 (14.1)	25 (10.4)	122 (15.3)			
No change	315 (30.3)	62 (25.8)	253 (31.6)			

All data are represented as number and percentage. P-value has been calculated using the Chi-square test. \*\*Significant at p < 0.05 level.





Physical activity before and during coronavirus disease-19 curfew.

Figure 4



Specific supplement taken during coronavirus disease-19 curfew.

### Figure 5



Domains	Overall [n (%)] (N=1040)	Male [n (%)] (N=240)	Female [n (%)] (N=800)	P value
Anxiety level				
Anxious	204 (19.6)	49 (20.4)	155 (19.4)	0.722
Not anxious	836 (80.4)	191 (79.6)	645 (80.6)	
Severity of anxiety				
Normal	836 (80.4)	191 (79.6)	645 (80.6)	0.669
Mild	61 (5.9)	12 (5.0)	49 (6.1)	
Moderate	100 (9.6)	25 (10.4)	75 (9.4)	
Severe	30 (2.9)	07 (2.9)	23 (2.9)	
Extremely severe	13 (1.3)	05 (2.1)	08 (1.0)	
Depression level				
Depressed	184 (17.7)	43 (17.9)	141 (17.6)	0.917
Not depressed	856 (82.3)	197 (82.1)	659 (82.4)	
Severity of depression				
Normal	856 (82.3)	197 (82.1)	659 (82.4)	0.259
Mild	96 (9.2)	17 (7.1)	79 (9.9)	
Moderate	71 (6.8)	20 (8.3)	51 (6.4)	
Severe	17 (1.6)	06 (2.5)	11 (1.4)	
Extremely severe	0	0	0	
Stress level				
Stressed	97 (9.3)	27 (11.3)	70 (8.8)	0.243
Not stressed	943 (90.7)	213 (88.8)	730 (91.3)	
Severity of stress				
Normal	943 (90.7)	213 (88.8)	730 (91.3)	0.485
Mild	59 (5.7)	17 (7.1)	42 (5.3)	
Moderate	38 (3.7)	10 (4.2)	28 (3.5)	
Severe	0	0	0	
Extremely severe	0	0	0	

Table 5 Prevalence of anxiety, depression, and stress using DASS-21 in relation to gender (n=1040)

All data are represented as number and percentage. Significant P-value has been calculated using Chi-square test.

of the participants, respectively, however, the results revealed that there was no significant relationship with gender in anxiety (P=0.669), depression (P=0.259), and stress (P=0.485).

# Discussion

The current study focused on describing the change in adults' nutritional and psychological behaviors during the COVID-19 curfew as compared with before the curfew in Saudi Arabia. In general, this study has found that the curfew has negatively affected nutritional behavior, psychological behavior, PA level, and weight status among Saudi adults [19].

The current findings showed that the participants reported an increase in the number of meals, snacks, and portion sizes during the COVID-19 curfew than before, supporting the results as they declared that the amount of food consumption and the frequency of snacking increased, and the participants who eat five meals per day increased from 19.9% to 31.1%. This may be due to boredom or the impact of the COVID-19 curfew on the psychological status as it makes people feel mentally stressed and anxious; thus, food consumption becomes a way to escape from negative emotions [19].

Considering WHO recommendations on eating at home will reduce contact with other people and lower the chance of being exposed to COVID-19 [20]. Therefore, during the curfew, most of the participants engaged in cooking more than before and reduced their fast-food consumption [5], which agreed with this study. Previous studies indicated that limited accessibility of food, limited opening hours of the grocery store, and having more time for meal preparation promoted cooking at home prominently. In addition, this study showed cooking participation was more evident among female participants by 67.0%. This may be due to the cultural norms in the Saudi population, as cooking chores are associated more with females. However, people might believe they consume a healthy diet as they shifted to home-cooked meals and reduced fast foods, but this does not always prove to be true because certain home-cooked meals are high in sugar and fat [19].

Regarding the changes in food groups, the results showed an increase in grain intake among the

participants. People tend to eat carbohydrates during the curfew since it increases serotonin production, which can work as antidepressive to positively influence mood status [21]. The increase was especially among male participants by 56.4%. A study in Italy agreed with this finding; however, the increase was evident in female participants [22]. While regarding meat intake during the curfew, the participants reported a significant difference between the sexes. Male participants reported a change of 56.6% with an increased intake of 40.8%, while female participants reported a total change of 46.9% and an increase of 30.7%. This could be explained as males naturally tend to consume meat more than females [23]. However, studies conducted in the Saudi population reported no change in meat intake among their participants [5,24].

In addition, the results showed a change in caffeinated drink consumption. The participants reported an increase in coffee and tea consumption during the curfew. A study from China agreed with these findings [25]. The change in tea consumption among male participants was higher than females by 10% when the differences in accordance with gender were measured. Major tea components such as polyphenols showed antiviral properties by inhibiting virus replication and adsorption [26].

People looked for alternative options to enhance their immune system and prevent the infection's progression as well [27]. The belief in the importance of nutritional supplements in fighting infections significantly increased during the pandemic [28]. This study's findings showed that almost half of the participants who were taking nutritional supplements reported an intake of vitamin-C supplement, followed by the intake of vitamin D in a quarter of the participants, similar to the results [5]. In fact, vitamins C and D have a vital role in supporting different immune cellular functions, antioxidant, and antiviral properties [29]. Although some vitamins and minerals have positive effects COVID-19 related to [30,31];there is no substantial evidence suggesting single any supplement treating or preventing viral infections [5].

Regarding immune-boosting foods, the current study indicated an increase in the use of honey, citrus fruits, ginger, and garlic with 59.2, 53.9, 46.8, and 41.2%, respectively among the participants. Our results are in line with [27–32]. Several pieces of evidence showed that some fruits, vegetables, spices, and herbs could lower the risk or severity of a wide range of viral infections due to their antiviral and antioxidant functions [33]. Thus, maintaining an adequate intake of micronutrients, a balanced diet, and a healthy lifestyle are useful strategies in guaranteeing a strong immune system and avoiding infection complications [11,33].

Isolation and curfew have led to changes in daily lifestyle habits, including PA. Recent studies from various countries showed a reduction in PA levels in male and female participants during the curfew [4,13,34]. The current study found a significant difference in PA patterns among male and female participants, and a significant decrease was noticed among males than females by 18.8%. In addition, males reported a decrease in their PA level by 44.2%, while females by 25.4%. In agreement with the present results, a study from the United Arab Emirates indicated a decrease in PA during the curfew was observed more in the male group than the female group [13]. Moreover, it was observed that during the curfew, 54.4% of the participants did not meet WHO recommendations regarding PA as compared with 31.5% before the curfew. A study conducted in Saudi Arabia showed similar results [12]. This could be because the closure of fitness centers, outdoor activities and parks, and overall movement limitation has decreased the ability to engage in PA accompanied by an increase in sedentary habits related to curfew, including telework and distance learning.

Due to insufficient PA and negative alterations in dietary habits, weight gain was observed during the curfew. This study's findings declared an increase in weight gain among the participants, especially among males by 63.8%. Data from the United Arab Emirates showed an agreement with the current result [11]. The reasons for weight gain might be limited PA along with increased consumption of coffee, dessert, salty snacks, and grain during the curfew. The increasing consumption of comfort food was evident in this study as well. Lippi and others [35] supported these results by declaring that unhealthy dietary habits were expected during the curfew, including an increase in snacking and coffee consumption and a reduction in fruits and vegetable intake.

Regarding psychological status during the curfew, in the current study, the prevalence of moderate to extremely severe anxiety, stress, and depression during the curfew was noted to be 13.8, 3.7, and 8.4%, respectively. The respondents reported experiencing less anxiety and stress compared with other studies [15,36]. The differences may result

from the following explanations: first, as the psychological effect of the pandemic became obvious, the National Center for Mental Health Promotion recognized the importance of early intervention and raising awareness to deal with emerging issues, for example, providing free counseling through hotlines (qariboon) or application, live streams on social media about the mental health issues related to the pandemic [37]. Second, this study was conducted after the curfew period when new COVID-19 cases dropped, which relieved psychological stress and decreased anxiety and depression symptoms in return. Although the prevalence of psychological symptoms is still small in the many studies, existing literature suggests greater symptoms requiring intervention and support, especially in the vulnerable population [38].

In addition, this study presents no significant relationship between psychological status and gender. This finding is inconsistent with numerous studies, which show a relationship between gender and psychological status [15,39,40]. Being female is a risk factor for experiencing psychological stress. In selfreported stress, sex differences are further reflected in the perceived need for psychological support services, which are often most evident in females [40].

## **Conclusion and future direction**

This study revealed that the COVID-19 curfew negatively affected the nutritional behaviors, psychological behaviors, PA, and weight status among Saudi adults. However, maintaining a balanced diet, ensuring adequate PA, and good mental health are effective strategies to guarantee good health. Therefore, public health officials should focus on increasing awareness of healthy eating and emphasizing the message 'to be physically active' to avoid negative consequences of the curfew and prevent weight gain. Moreover, public health interventions are needed to address parenting-specific stressors, develop useful strategies for coping with parenting difficulties to reduce the effect of this pandemic on adults' mental health.

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# **Conflicts of interest**

There are no conflicts of interest.

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# Predictors of macrovascular complications in Al-Madinah Al-Munawara type 2 diabetes mellitus patients, Saudi Arabia: A cross-sectional study

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### Background/aim

Diabetes mellitus is a metabolic disease characterized by chronic hyperglycemia, which causes macrovascular complications that account for most of the morbidity, hospitalizations, and deaths. The goal of this study is to evaluate the relationship between macrovascular complications and associated risk factors in Al-Madinah Al-Munawara patients suffering from type 2 diabetes mellitus.

### Patients and methods

This descriptive cross-sectional study involved 275 type 2 diabetes mellitus patients who resided in Al-Madinah Al-Munawara and visited the diabetic clinic at King Fahd Hospital on a regular basis. Participants in the study ranged in age from 20 to 65. Hemoglobin, glycated hemoglobin, serum calcium, fasting blood glucose, and lipid profile were analyzed.

### Results

Out of 275 patients with type 2 diabetes mellitus, 113 have had macrovascular complications. A significant association (P<0.05) has been observed between the age of the patients and different forms of macrovascular complications, which impact 62.7% of the elderly (41–65 years old). The prevalence of macrovascular complications was higher among females. Glycated hemoglobin levels positively correlated with the number of hospitalizations, high blood pressure, hemoglobin, and serum cholesterol levels. Macrovascular complications are positively correlated with age, sex, hypertension, and therapy type (P<0.05), while physical activity has a negative significant effect on the appearance of macrovascular complications by multiple regression analysis.

### Conclusion

In this study, diabetic macrovascular complications were prevalent. The age, sex of participants, and type of treatment were independent predictors of macrovascular complications. Hypertension has a significant positive effect on the appearance of macrovascular complications. Physically inactive patients have a higher risk of macrovascular complications.

### Keywords:

Al-Madinah Al-Munawara, macrovascular complications, type 2 diabetes mellitus

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# Introduction

Diabetes mellitus is not merely a disorder of carbohydrate metabolism but a cause of vascular disease affecting nearly all blood vessel types and sizes. Chronic hyperglycemia occurs because of relative or absolute insulin deficiency in adults and is associated with an unhealthy diet, lifestyle, physical inactivity, and obesity, which lead to macrovascular complications. Indeed, vascular complications are responsible for the morbidity, hospitalizations, and deaths that occur in diabetic patients [1].

Type 2 diabetes mellitus usually develops when the body's cells become resistant to the action of the hormone insulin. The pancreas will produce more insulin to overcome this resistance and lower blood sugar levels. Over time, the pancreas will lose its ability to produce insulin. As a result, glucose builds up in the bloodstream instead of being transported to cells where it is used for energy, resulting in a rise in blood sugar levels [2].

In 2019, type 2 diabetes was estimated to impact 463 million people worldwide [3]. It is characterized by insulin resistance, decreased insulin production, and

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eventually pancreatic beta cell loss [4]. Insulin resistance increases glucose synthesis in the liver and decreases glucose absorption in muscle, liver, and adipose tissue, leading to decreased blood flow to the liver, muscle cells, and fat cells [5].

The long-term complications of type 2 diabetes can be divided into two types: microvascular and macrovascular. Because individuals with type 2 diabetes can be presymptomatic for years, they are at higher risk of developing both types of complications, which will worsen the disease and affect the patient's well-being, quality of life, daily activities, and socioeconomic status, thereby increasing morbidity and mortality [6].

Macrovascular complications can impact patients with type 2 diabetes in several ways. Coronary heart disease, arrhythmias, cardiomyopathy, sudden death, cerebrovascular disease, and peripheral artery disease are all results of macrovascular impairment that impacts larger blood vessels. Arrhythmia is a disorder of the heart's rhythm, whereas cardiomyopathy is a heart muscle disease [6,7]. Cardiovascular disease is the leading cause of death among people with diabetics. Finally, cerebrovascular disease affects the brain's blood vessels and blood flow [7]. Early macrovascular complications are associated with atherosclerotic plaque in the vasculature, which supplies blood to the brain, heart, limbs, and other organs [8]. Myocardial infarction, angina pectoris, and stroke are all concerns associated with latestage macrovascular disease, which involves full obstruction of the vessels [9].

The development of macrovascular problems in type 2 diabetes is linked to several risk factors. These factors could be grouped as sociodemographic factors (age, sex, and marital status), behavioral factors (obesity, diet), and clinical factors (durability of diabetes (years), glycated hemoglobin (HbA1c) (mmol/mol), duration of diabetes, and hypertension) [7,9]. According to specific studies, factors such as sex, age, marital status (single or divorced), family history of diabetes mellitus, longer duration of diabetes, hypertension, obesity, poor glycemic control, adherence to diet, mixed medication, and insulin therapy alone were predictors of macrovascular complications among type 2 diabetes mellitus patients [10]. The healthcare system in the kingdom has improved more than before, leading to optimal healthcare for diabetic patients. Therefore, there has been a decrease in mortality rates and length of stay in hospitals [11].

This study aims to determine the relationship between macrovascular complications and associated risk factors in patients in Al-Madinah Al-Munawara, Saudi Arabia, who have type 2 diabetes mellitus.

# Patients and methods Patients and study design

Between October 2021 and January 2022, an observational, descriptive, cross-sectional survey was created on 275 patients aged from 20 to 65 years old with type 2 diabetes mellitus.

The participants were recruited through an online survey circulated on social media platforms such as WhatsApp and Twitter in Al-Madinah Al-Munawara, Saudi Arabia. The data collected through an online questionnaire consisted of 37 questions that included a detailed personal and medical history of the last 3 months. Next, cases are interviewed at King Fahd Hospital's diabetes clinic. Type 2 diabetes clients visiting the hospitals regularly (every 1 month) as outpatients and available during the data collection period were participants and eligible for this study.

Data related to medical examinations, BMI, and laboratory investigations for the patients with type 2 diabetes were collected from their files and during the personal interview in the diabetes clinic at King Fahd Hospital.

Inclusion criteria included type 2 diabetic patients living in Al-Madinah Al-Munawara aged 20–65 years old. Out of 400 patients with incomplete data, patients suffering from the coronavirus disease of 2019, chest infections, cancer, or pregnant women were excluded.

## Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patients have given their consent for their images and other clinical information to be reported in the journal. The patient understands that his name and initials will not be published and due efforts will be made to conceal her identity, but anonymity cannot be guaranteed.

## **Ethical consideration**

The present study obtained the ethical approval to start this study from the Ethical Committee of the College of Applied Medical Sciences at Taibah University with approval number 2021/107/203 CLN. We clarified that participation is optional and that all participants
are informed they have the right to withdraw from the study at any time before they fill out the questionnaire. The participants' privacy is protected by not mentioning any private information in the study.

#### Sample size calculation

The targeted sample size for this study was 60 male participants and 60 female participants, as estimated by Epi Info (Epi Info, v. 7.2.4.0; CDC, Atlanta, Georgia, USA) with alpha 0.05, beta 0.10, and a 45% expected difference between males and females, determined based on a two-sided test. The targeted individuals reside in Al-Madinah Al-Munawara. The study population included type 2 diabetic patients aged 20–65 years old.

#### Methods

The variables under study were sex, age, income, duration of illness, family history of diabetes, physical exercise regimen, BMI, type of treatment, presence of other comorbidities, blood pressure, hemoglobin level, HbA1c level, and serum lipid profile [e.g. low-density lipoprotein (LDL), highdensity lipoprotein (HDL), cholesterol].

#### Data collection

The tool used for data collection was a questionnaire. A phone number was necessary in the event of follow-up questions. All the questions were translated from English into Arabic and back translated from Arabic into English by two bilingual experts. The questionnaire consisted of 37 questions. The questionnaire was used to collect information on sex, age, duration of illness, family history of diabetes, hospitalization, hospital admission for diabetic ketoacidosis with coma, cardiovascular disease, presence of macrovascular complications, physical activity and patterns of exercise, BMI, presence of other comorbidities, frequency of urination, blood pressure.

The last week's follow-up results related to laboratory investigations for the patients with type 2 diabetes were collected from their files on the computer in the diabetes clinic at King Fahd Hospital. It included HbA1c level, fasting blood glucose, lipid profile (e.g. LDL, HDL, triglycerides, cholesterol), and serum calcium level. The total serum cholesterol level was considered elevated at 200 mg/dl and the triglycerides level was elevated at 150 mg/dl. HDL cholesterol was considered low at 40 mg/dl in males and less than 50 mg/dl in females. LDL cholesterol levels were elevated at 100 mg/dl [12].

#### Routine analyses

Venous blood samples were withdrawn from all participants after an overnight fast, and the separated sera were stored at -20°C. Fasting serum glucose was measured using standard colorimetric procedures. Serum triglycerides, cholesterol, HDL, and LDL were measured using the colorimetric method using the bio-diagnostic kit. HbA1c was measured using ion exchange chromatography on a D-10 system. The total Bio-Rad calcium concentration was measured using atomic absorption spectrophotometry. The hemoglobin concentration is then determined by the result produced by the photoelectric colorimeter.

#### Statistical analysis

The statistical analysis of the collected data was carried out using the Statistical Package for the Social Sciences, version 28 (SPSS, Chicago, IL 60606-6307). The variables were categorized according to independent variables, including sociodemographic characteristics such as age, sex, BMI, disease duration, and the HbA1c level, and dependent variables, including macrovascular complications of type 2 diabetes mellitus. The association between disease characteristics (i.e. age, sex, disease duration, glycemic control, BMI, and hypertension) and participants' sociodemographic characteristics was examined using the  $\chi^2$  test. Spearman's correlation analysis was conducted between the HbA1c level and continuous variables in the study. The strength of the correlation is described as follows: 0.0–0.19 'very weak,' 0.20-0.39 'weak,' 0.40-0.59 'moderate,' 0.60-0.79 'strong,' and 0.80-1.0 'very strong' [13]. relationship between To evaluate the the independent and dependent variables, a multiple logistic regression test was used. A P value of 0.05 was used to determine the significance of associations.

#### Results

Out of 275 patients with type 2 diabetes mellitus, 113 patients have had macrovascular complications. Table 1 shows the results of  $\chi^2$  tests for associations between sociodemographic, clinical, and laboratory characteristics of participants with macrovascular complications. Age of the patients is statistically significantly associated with macrovascular problems (P=0.002). Macrovascular problems impact 37% of the younger (20-40 years old) and 62.7% of the elderly (41-65 years old). Sex and macrovascular problems are statistically significantly associated (P=0.035).Macrovascular complications affect 61.7% of female patients compared to 47% of male patients.

Macrovascular complications and a family history of diabetes are statistically significantly associated (P=0.042). It impacts 60.4% of individuals with a positive family history of diabetes mellitus. Physical activity and macrovascular complications are statistically significantly associated (P=0.027). Sixty-four percent of people who do not exercise have macrovascular problems. Hypertension and

macrovascular problems are statistically significantly associated (P<0.001), and 78.4% of the patients with hypertension have macrovascular complications (Table 1).

Macrovascular problems affect 79.3% of patients with anemia (hemoglobin>12 g/dl). Hemoglobin and macrovascular complications are statistically

Table T Socioucinourabilic, cilincal, and iaboratory characteristics of barticibalits with types of macrovascular complicat	e 1 Sociodemographic, clinical, and laboratory characteristics of participants with types of macrovascular com	plications
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Variables	Categories	Macrovascular
		complications (113)
		[ <i>n</i> (%)]
Age (in years)*	20–40 years	17 (37.0)
	41–65 years	96 (62.7)
Sex*	Male	31 (47.0)
	Female	82 (61.7)
Education	No read or write	20 (74.1)
	Primary, secondary	21 (58.3)
	High school	19 (61.3)
	Certificate and above	53 (50.5)
Income	Low <3000	23 (60.5)
	Moderate 3000–10000	54 (56.3)
	Hign >10 000	36 (55.4)
Family history of diabetes*	Yes	93 (60.4)
	NO	20 (44.4)
Smoking history	Yes	14 (66.7) 00 (55.6)
	NO	99 (55.6)
Physical activity*	Yes	49 (49.5)
214	NO	64 (64.0)
BMI	Normal	20 (43.5)
	Obese	35 (56.5) 58 (63.7)
Turne of tweetment	Obese Orel humanhumania theranu	58 (65.7)
Type of treatment	Oral hypoglycemic inerapy Oral hypoglycemic and insulin therapy (mixed therapy)	36 (50.3) 34 (50.0)
Duration of DM		52 (58 4)
	>5 years	61 (55.5)
Hypertension*	Yes	69 (78.4)
	No	44 (39.6)
Serum cholesterol	<200 mg/dl	31 (66.0)
	≥200 mg/dl	20 (55.6)
Serum HbA1c	Accepted control	56 (57.7)
	Uncontrolled	11 (42.3)
Serum triglyceride	Less than 150 mg/dl	23 (56.1)
	More than 150 mg/dl	15 (60.0)
Serum HDL	Less than 40 mg/dl	25 (62.5)
	More than 40 mg/dl	11 (55.0)
Serum LDL	Less than 100 mg/dl	19 (54.3)
	More than 100 mg/dl	16 (69.6)
Serum hemoglobin*	Less than 12 g/dl	23 (79.3)
	12–15 g/dl	23 (52.3)
Serum calcium	Less than 9 mg/dl	18 (69.2)
	More than 9 mg/dl	10 (50.0)
Hospital follow up	I have not seen a doctor in the last year	24 (60.0)
	From 1–2	27 (49.1)
	From 2–5	40 (67.8)
I have the hard a stand in the second Frances		∠∠ (48.9)
Hospital admission in the past 5 years	res No	35 (59.3) 78 (55.7)
Number of boositol admissions in the last of a	No	70 (00.7)
Number of nospital admissions in the last year		80 (55.6)
	2 times and above	20 (00.9) 5 (55 B)
Hospitalized due to diabetic same		5 (30.0)
i lospitalized due to diadelic coma	No	108 (58 1)

\*Significant association with macrovascular complications at (P>0.05) using  $\chi^2$  test.

significantly associated (P=0.017). The remaining factors do not appear to be associated with macrovascular complications in any way that is statistically significant (P>0.05). These factors include education level, income, history of smoking, treatment type, duration of diabetes, BMI, dyslipidemia, HbA1c, serum calcium, number of hospital admissions in the past year, and hospitalization due to diabetic coma, as shown in Table 1.

Table 2 displays the results of Spearman's correlation analysis between the HbA1c level and continuous

Table 2 Spearman's correlations between continuous variables and glycated hemoglobin level of participants

Variables	Correlation coefficient	P value
Age	0.057	0.344
Duration of DM	-0.058	0.337
BMI	-0.014	0.816
Serum cholesterol	0.343	<0.001**
Hemoglobin	0.281	<0.001**
High blood pressure	0.156	0.010*
Number of hospitalizations	0.119	0.049*

DM, diabetes mellitus; HDL, high-density lipoprotein; LDL, lowdensity lipoprotein. \*Significant difference at *P* value less than 0.05. \*\*Highly significant difference at *P* less than or equal to 0.01. variables in the study. The strongest association was observed between the serum cholesterol level and the HbA1c level (r=0.343, P<0.001), then hemoglobin (r=0.281, P<0.001), then blood pressure (r=0.156, P=0.010). The least significant relationship was the number of hospitalizations (r=0.119, P=0.049). The findings suggest that an elevated level of blood pressure, cholesterol, and hemoglobin corresponds to an elevated HbA1c level (>10). Otherwise, the association between the HbA1c level and other variables such as age, duration of illness, and BMI is not statistically significant (P>0.05).

Table 3 displays the findings of  $\chi^2$  tests for an association between sex and macrovascular complications. A statistically significant correlation (P < 0.05) has been shown between sex and macrovascular complications. Females experienced macrovascular complications at a higher rate than males (P=0.049). Other than that, peripheral artery disease (diabetic foot), cerebrovascular disease, or cardiovascular disease do not differ statistically significantly (P>0.05) between males and females.

Table 4 presents the results of multiple logistic regression tests performed to detect the relationships

Variables	All diabetic patients [n (%)]	Macrovascular complications [n (%)]	Cardiovascular disease (coronary artery diseases, arrhythmia) [ <i>n</i> (%)]	Cerebrovascular disease (stroke) [n (%)]	Peripheral artery disease [n (%)]
All patients	275 (100)	113 (100)	78 (100)	8 (100)	27 (100)
Male	90 (32.7)	31 (47)	26 (33.3)	1 (12.5)	10 (27)
Female	185 (67.3)	82 (61.7)	52 (66.7)	7 (87.5)	17 (63)
P value	0.888	0.049*	0.893	0.216	0.615

Table 3 Prevalence and association between sex and different types of macrovascular complications in the studied sample

HbA1c, glycated hemoglobin. \*Significant association with macrovascular complications at P value more than 0.05 using  $\chi^2$  test.

able 4 Multiple logistic regressions for	factors associated with the	e occurrence of macrovascular	complications
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Variables		В	SE	Wald	DF	Significance	Exp(B)	95% con interval fo	nfidence or Exp( <i>B</i> )
								Lower bound	Upper bound
	Intercept	-1.482	1.741	0.725	1	0.395			
	Age	-0.942	0.444	4.491	1	0.034*	0.390	0.163	0.932
	Sex	-0.930	0.370	6.337	1	0.012*	0.394	0.019	0.814
	BMI	-0.008	0.021	0.130	1	0.719	0.992	0.952	1.034
	Duration of DM	0.472	0.370	1.634	1	0.201	1.604	0.777	3.310
	Serum HbA1c (past 3 month)	0.448	0.272	2.715	1	0.099	1.565	0.919	2.665
	Hypertension	2.092	0.386	29.398	1	<0.001**	8.105	3.804	17.267
	Dyslipidemia	0.148	0.458	0.105	1	0.746	1.160	0.473	2.846
	Education	0.141	0.170	0.686	1	0.407	1.152	0.825	1.608
	Type of treatment	0.690	0.275	6.297	1	0.012*	1.993	1.163	3.415
	Physical activity	-0.720	0.352	4.191	1	0.041*	0.487	0.024	0.970

DM, diabetes mellitus; HbA1c, glycated hemoglobin. \*Significant difference at P value less than 0.05. \*\*Highly significant difference at P value less than or equal to 0.01.

between risk factors associated with the occurrence of macrovascular complications. The results found that macrovascular complications are positively correlated with age, sex, hypertension, and therapy type (P < 0.05), while physical activity has a significant negative effect complications macrovascular (P=0.041).on Macrovascular diabetes complications are more common among females. Age (B=-0.942, P=0.034) indicates that the higher the age, the higher the macrovascular complications are. Macrovascular complications are more common in diabetics who additionally use insulin therapy, oral hypoglycemic medications (B=2.092, P=0.001), and suffer from hypertension (B=0.69, P=0.012). Finally, physical activity has indicated that the greater the activity, the fewer macrovascular complications occur.

#### Discussion

The prevalence of type 2 diabetes mellitus has increased rapidly around the world. Chronic diabetes is associated with a high rate of morbidity and mortality, with more than four million people aged 20-79 dying from diabetes-related disorders. Saudi Arabia is one of the top 10 nations where diabetes is most prevalent [14]. According to the International Diabetes Federation, the prevalence of type 2 diabetes mellitus in adults has increased from 366 million in 2011 to 536 million in 2021. By 2030, researchers expect that 643 million people will have this condition. In the Middle East, there are 73 million individuals with type 2 diabetes mellitus, and this number is expected to rise to 95 million by 2030. In Saudi Arabia, type 2 diabetes mellitus affects four million people, with an expected five million by 2030 [6].

In Saudi Arabia, diabetes mellitus is increasing faster and becoming a dangerous medical problem associated with higher morbidity and mortality [14]. Our study included 275 patients with type 2 diabetes mellitus. More than half of the patients (71.3%) were between 41 and 65. Females represented 67.3% of the study sample. Compared to 37% of the younger age group, between 20 and 40 years old, 62.7% of the older age group (41–65 years) experienced macrovascular complications.

To our knowledge, this is the first study done in Al-Madinah Al-Munawara to evaluate the relationship between macrovascular complications and associated risk factors in patients with type 2 diabetes mellitus.

In our study, females had a larger prevalence of macrovascular problems than males, with 61.7%

against 47%. In contrast to the Upper Egypt study conducted by Hussein and Menasri [6], they found that 60% of males had a higher cardiovascular disease risk factor than females; this difference was related to smoking, physical activity, and family history.

In our study, those who did not engage in any form of physical exercise (64%) had a higher risk of getting macrovascular complications, whereas those who were physically active (49.5%) had a lower risk. In a study conducted in Upper Egypt, only 98 (12.25%) of 800 individuals were physically inactive. The difference is justifiable since the major economic activity in the villages is farming [15]. In our study, 78.4% of the patients with hypertension had macrovascular complications.

The present study reported that HbA1c and serum total cholesterol showed a strong positive correlation. Consequently, a larger distribution of atherogenic cholesterol is linked to abnormal glycemic control, higher weight, and greater insulin resistance in both men and women with diabetes mellitus [15], which explains the relationship between serum total cholesterol and glycemic control. Furthermore, there exists a substantial correlation between elevated HbA1c levels and elevated blood pressure. Peng *et al.* [16] supported our results. Individuals with higher HbA1c were found to have high blood pressure and higher lipid profiles as measured by LDL cholesterol and total cholesterol. Results from Blecker *et al.* [17] corroborate this conclusion.

A possible explanation for the significant correlation between hyperglycemia and hypertension could be the existence of common risk factors, particularly obesity. In addition, the emergence of hyperglycemia and hypertension are both influenced by inflammatory processes [18]. According to our analysis, patients with higher HbA1c values experienced noticeably more hospitalizations. Findings from Blecker *et al.* [17] corroborate this conclusion.

In the present study, out of 275 patients with type 2 diabetes mellitus, 41% had macrovascular complications. Females were more likely than males to have macrovascular problems, with 61.7% compared to 47%. However, our percentage is higher and opposite to the study in Saudi Arabia, where macrovascular complications were more common in males (5.8%) than in females (2.8%) [19].

Multiple logistic regression analyses were used to investigate the factors associated with the appearance

of macrovascular complications. According to our development macrovascular study, the of complications was significantly positively impacted by older age, female sex, hypertension, and kind of treatment (P < 0.05). This is in line with a study done in Riyadh by Alaboud et al. [14], which revealed that females had a higher risk of macrovascular complications than males and that these complications increased with age.

The macrovascular complications affected 62.7% of the older adults aged 41–65 years; this might be related to a long history of smoking and high cholesterol, hypertension, or obesity [7]. Females have a higher prevalence of macrovascular complications than males due to high cholesterol levels and obesity [20]. Diabetic patients with macrovascular issues are hypertensive because hypertension is linked to an increased risk of heart disease and stroke [21].

According to our study, physical activity has a significant negative effect on macrovascular function (heart and coronary artery health). The macrovascular incidence rate remained higher in less active females. A study done in Egypt by Hussein *et al.* [15] shows that physically inactive patients have a higher risk of cardiovascular disease, and this agrees with our study. First, it has been suggested that physical activity increases insulin sensitivity. Second, physical activity has also been found to reduce intraabdominal fat, a known risk factor for insulin resistance [22].

#### Conclusion

This study is the first of its kind in Al-Madinah Al-Munawara and provides insight into the severity of macrovascular complications in type 2 diabetic patients. The findings indicated that macrovascular complications were a major cost to the nation. The chance of developing macrovascular complications is increased when other chronic medical conditions, such dyslipidemia, ischemic heart disease, as and hypertension, coexist. Our results showed that the age of participants, sex, and type of treatment were independent predictors of macrovascular complications. Hypertension has a significant positive effect on the appearance of macrovascular complications. Physically inactive patients have a higher risk of macrovascular complications.

#### Recommendation

Increased knowledge and focus on the macrovascular complications that type 2 diabetics may face are needed

in our area. Aggressive health education and promotion programs, along with improved public health awareness services, will lower the nation's fatal macrovascular consequences from diabetes mellitus.

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#### **Conflicts of interest**

There are no conflicts of interest.

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### Bcl-2 expression and image analysis of premalignant and malignant colonic lesions Sonia L. El-Sharkawy, Naglaa F. Abbas, Wafaa E. Abdelaal, Manal A. Badawi

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#### Background/aim

Colorectal cancer is the third malignancy worldwide with high mortality. The development of colorectal carcinoma is a multiple step process that turns normal cells into malignant ones. One of these steps is inhibiting apoptosis. Bcl-2 is the key regulators of apoptosis and thus inhibits programmed cell death. The relationship between cell death and cell proliferation is balanced through apoptosis. This study aimed to evaluate immunohistochemical bcl-2 expression, nuclear morphometric parameters, and cell cycle values in premalignant and malignant colon lesions.

#### Material and methods

Sixty colonic paraffin blocks (10 normal mucosa, 20 adenomas, and 30 carcinomas) from private laboratories and the Pathology Department, National Research Centre, Cairo, Egypt, were included in this study. Bcl-2 expression was evaluated by immunohistochemistry. Nuclear morphometric parameters and cell cycle values were studied using an image analysis system.

#### Results

Immunohistochemical results showed expression of bcl-2 in the lower half of normal colonic crypts. Bcl-2 positivity was detected in 53% of carcinomas and 85% of adenomas with significant difference. The percentage of bcl-2 positive cells in carcinomas was significantly decreased with increasing grades. In carcinomas, nuclear area showed significant increase with increasing grades. Nuclear area showed significant difference between high-grade dysplastic adenomas and carcinomas. Carcinomas showed high proportion of aneuploid cells with significant difference than adenomas. Inverse correlation was detected between aneuploidy and bcl-2 positivity.

#### Conclusion

Bcl-2 protein has a role in early event of colorectal carcinogenesis. The acceptable reliability of immunohistochemical, nuclear area, and cell cycle analysis may serve as diagnostic and prognostic indicators in benign and malignant colorectal lesions.

#### Keywords:

Bcl-2, colonic carcinoma, image analysis, immunohistochemistry

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#### Introduction

Colorectal cancer (CRC) is the third malignant tumor with high mortality accounting for about 881.000 deaths worldwide [1,2]. This tumor is the second cancer in women and the third in men [3]. Its incidence in developed countries is increasing among persons less than 50 years old [4]. Limited clinical signs are demonstrated in early stages of CRC and patients are diagnosed with metastasis, rendering difficult therapy, therefore early diagnosis is a challenge [5,6].

Bcl-2 family of proteins which regulate apoptosis, and disturbance of this regulation can lead to many pathological consequences including formation of malignant tumors [7]. The antiapoptotic bcl-2 gene is 26-kD protein which blocks apoptosis and controls the release of preapoptotic factors. These factors are responsible for stabilizing the mitochondrial membrane and activation of caspase [8].

Aberrant expression of bcl-2 is closely related to carcinogenesis in most malignant tumors, including CRC [8]. Bcl-2 family members are classified into antiapoptotic/prosurvival three subgroups: the the proapoptotic proteins, and the proteins, multidomain proapoptotic proteins [9]. The proapoptotic proteins are characterized by the presence of the N-terminal BH4 domain. This domain can bind other proteins which are not from bcl-2 family proteins, forcing them to play a role in inhibiting apoptosis by functions such as, autophagy, proliferation, DNA repair differentiation, and angiogenesis [10]. The antiapoptotic protein group makes their prosurvival role by binding and

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inhibiting the proapoptotic protein which are the effectors of apoptosis and the sensors of cellular stress [11].

Aberrant expression of bcl-2 proteins has been detected in malignant tumors from different origins. Other studies have shown that high levels of antiapoptotic proteins have an action in modulating apoptosis, tumor initiation, response to therapy, and progression [10,12]. Several studies detected that bcl-2 expression associated with is cancer progression including colon cancer. Some studies found a correlation between Bcl-2 expression and better prognosis [13,14]. Others demonstrated that bcl-2 was a bad prognostic for patients having malignant tumors [15–17].

Morphometry has a great role in many areas of diagnostic histopathology. Computer-assisted image morphometric analysis has been introduced to quantitate various grading systems. Increasing abnormalities in morphology of nuclei correlates with the advanced grade of tumor as well as poor prognosis in many cancers. Nuclear morphometry correlates with mitotic activity, tumor size, and lymph node metastasis. Nuclear morphometry can be added to clinical and histopathological features and used to prognosticate and classify cancer cases into low-risk and high-risk groups [18].

Cellular growth and cell preparation for division between cell divisions is called the cell cycle. The cell cycle is divided into four phases; the length of cell cycle phases is an important characteristic of cell life. They are the G1 (gap 1), S (DNA synthesis), G2 (gap 2), and M (mitosis) phases [19].

Information about cell cycle is very helpful for predicting tumor development. Cancer cells show unscheduled proliferation, genomic instability, and chromosomal instability [20]. Dysregulation of the cell cycle is the first step in carcinogenesis, tumor invasion, and metastases [21].

The progression of cells in the cell cycle has highly organized steps related to intracellular and extracellular signals and controlled by endogenous and exogenous factors. Various approaches are used to identify cell cycle phases. The most frequently used approach is based on the analysis of DNA. The cells in G1 and G0 have half DNA content as compared to G2 and M cells. The DNA histogram enables the estimation of the percent of cells in G1/G0, S, and G2/M phases [19].

This study aimed to evaluate immunohistochemical expression of bcl-2 and image analysis of nuclear parameters in premalignant and malignant tumors.

#### Material and methods Samples and the study design

The present study retrospectively obtained paraffin blocks and reviewed the database of 60 cases that underwent curative surgery for CRC from private laboratoriess and the Pathology Department, National Research Centre. The used material was derived from anonymous archival tissue samples embedded in paraffin blocks. So, no consent was obtained.

The 60 colonic paraffin blocks included in this study were 10 normal mucosa, 20 adenomas, and 30 carcinomas. The adenoma cases were 10 with lowgrade dysplasia and 10 with high-grade dysplasia. The carcinoma cases were 10 grade I adenocarcinoma, 10 grade II adenocarcinoma, and 10 grade III adenocarcinoma.

#### Inclusion criteria

Cases of CRC with no other cancers or chronic diseases and cases did not receive radiotherapy or chemotherapy.

#### **Exclusion criteria**

Cases with other cancers or received radiotherapy or chemotherapy and cases with chronic diseases were excluded from this study.

#### **Ethical consideration**

The present study was conducted with the Code of Ethics of the World Medical Association, according to the principles expressed in the Declaration of Helsinki. This study was approved by the Local Ethics Committee of National Research Centre, Cairo, Egypt with approval number 16/308. The used materials were derived from archived tissue samples embedded in paraffin blocks. The personal data of the patients were replaced by numerical codes for privacy and confidentiality.

#### Methods

Three sections  $(4 \,\mu m \text{ thick})$  were cut from each block. One section was stained with hematoxylin and eosin for histopathologic evaluation and morphometric analysis. The second section was stained with Feulgen stain for measurement of cell cycle parameters. The third section was mounted on a

positively charged glass slide for immunohistochemical staining using anti-bcl-2 antibody.

#### Immunohistochemistry

For immunostaining, the sections were deparaffinized and rehydrated through a graded series of alcohol. Endogenous peroxidase activity was blocked by freshly prepared 0.3% hydrogen peroxide in methanol for 20 min. Then microwave antigen retrieval was used, followed by incubation with bcl-2 antibody. The Ultravision LP polymer system (Lab Vision, Fremont, California, USA) and the chromogen diaminobenzidine were used to amplify and visualize the antigen-antibody complex. The expression of bcl-2 was evaluated in the entire section at a magnification of ×400. Bcl-2 showed cytoplasmic staining. The bcl-2 was determined as the percentage of positively stained cells to the total number of cells. The percentage positivity was graded as low (>25%), medium (25-50%), and high (<50%) [22].

#### Image analysis

The image analysis was performed at the Pathology Department, National Research Centre, using the Leica Qwin 500 Image analyzer (LEICA Imaging Systems Ltd, Cambridge, UK) which consist of a Leica DM-LB microscope with a JVC color video camera attached to a computer system.

#### Morphometric analysis

Nuclear morphometric analysis was performed on hematoxylin and eosin-stained slides. A 100-150 nonoverlapping nuclei were evaluated per case by a trained pathologist who was blinded of the histopathological diagnosis. Images were captured at ×400 magnification. Calibration was performed before each measuring session. Nuclei were outlined using the mouse of the computer. These outlines were refined automatically and automatic measurements were made by the software. After measurement, the data were transferred to the Excel sheet for further analysis. Nuclear morphometric parameters analyzed included nuclear area, diameter, perimeter, roundness  $(4\pi A/P^2)$ , texture (amount of local intensity variation in the digitized image of a nuclear profile) and mean grey (the sum of grey values for each pixel within the nucleus/number of pixels measured).

#### Cell cycle analysis

Two of the most popular cytometric applications are the measurement of cellular DNA content and the analysis of the cell cycle. The cell cycle profile was determined by staining the DNA with a specific dye and measuring its intensity. The Feulgen stain is not only specific for the localization of DNA in chromosomes, but at the same time the intensity of the reaction may be considered as an index of the amount of DNA present in the cell [23].

Analysis was performed first on the normal control specimens to determine the reference values. Selection of nuclear boundaries is usually performed automatically by the image analysis system; however, some degree of interaction or editing is usually needed for optimal nuclear selection 'Touching' nuclei can be 'Cut' from each other, and cellular fragments or extraneous cells can be erased prior to measurements. Only separate, intact nuclei were measured. Distorted or overlapping nuclei and nuclear fragments were manually eliminated from measurement. All these facilities were supplied as editing functions in the Leica Qwin 500 image analysis systems.

Care was taken to measure various nuclei representative of the examined lesion, so that measurements were not biased toward the degenerated, bizarre, or anaplastic nuclei. Each field was focused before the measurement to exclude cut nuclei and blurred ones. The optical density of the selected nuclei in each microscopic field is then measured and automatically converted by the system into cell phase in the life cycle. We select many fields until the desired number of nuclei 100-150 has been measured. Percentages of cells in the first growth phase (G1), proliferating cells (S), and second growth phase (G2) were calculated and determined automatically by the system. All collected data were stored to be reanalyzed. The proliferating cell percentage was calculated by summation of percentage of cells in synthesis phase (S) and cells in gap 2 (G2) phase of the cell cycle.

#### Statistical analysis

All data were collected, revised, coded, and entered into the Statistical Package for the Social Sciences (version 23.1; IBM SPSS, Armonk, New York, USA). The quantitative data were presented as mean±SD. The comparison between groups regarding quantitative data was done using the analysis of variance test. The qualitative variables were presented as number and percentages. The comparison between groups regarding qualitative data was done using the  $\chi^2$  test. *P* values of less than 0.05 were considered to indicate statistical significance.

#### Results

#### **Bcl-2** immunoreactivity

Normal colonic mucosa showed bcl-2 immunoreactivity in the lower half of colonic crypts.

The epithelial cells lining the upper half of the crypt and surface epithelium were negatively stained for bcl-2. Out of 20 adenoma cases, 17 (85%) cases showed positive staining for bcl-2. Nonsignificant correlation was detected between bcl2 expression and degree of dysplasia in adenomas where 90% of low-grade dysplasia and 80% of high-grade dysplasia were positively stained for bcl-2 (Table 1).

The carcinoma cases had a significantly lower rate of bcl-2 positivity than adenomatous cases where it was

detected in only 53.3% of cases (vs. 85% of adenoma cases). Bcl-2 was expressed in 80% of grade I, 50% of grade II, and 30% of grade III showing significant negative correlation between bcl-2 expression and tumor differentiation. Semiquantitative determination of the percentage of bcl-2 immunoreactive tumor cells revealed a significant difference between colonic adenomas and carcinoma where most of the positive adenoma (65%) showed high staining (<50%), while majority of the positive carcinoma (68.9%) displayed low immunoreactivity for

Table 1	Bcl-2	expression	in	studied	groups
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	Bcl-2 +ve [n (%)]	Bcl-2 -ve [n (%)]	Total
Adenomas			
Low grade dysplasia	9 (90)	1 (10)	10
High grade dysplasia	8 (80)	2 (20)	10
Total	17 (85)	3 (15)	20
Adenocarcinoma*			
Grade I	8 (80)	2 (20)	10
Grade II	5(50)	5 (50)	10
Grade III	3 (30)	7 (70)	10
Total	16 (53.3)	14 (46.7)	30

<sup>\*</sup>Significant difference at *P* value less than 0.05 using the  $\chi^2$  test.

#### Figure 1



Bcl-2 imunohistochemical expression; (a) normal colonic mucosa revealing positive expressin in lower half of colonic crypts (x200), (b) colonic adenoma showing high percetage of positively stained cells (x100), (c) grade I colonic adenocarcinoma showing moderate expression (x100), and (d) grade II colonic adenocarcinoma revealing negative expression (x200).

bcl-2 (>25%). Nonsignificant correlation was detected between the degree of dysplastic changes in adenoma and the proportion of positively stained cells. On contrary a significant inverse correlation was detected between the proportion of bcl-2 positively stained cell and histologic grades of carcinoma on comparing grade I and grade II with grade III. All cases of grade III and only 61.9 of grade I and grade II cases showed low staining. None of grade III cases and 15.5% of grade I and grade II cases showed high staining, as shown in Fig. 1 (Table 2).

#### Nuclear morphometry

Adenoma cases revealed significantly higher values of nuclear area than those of normal colonic mucosa (P>0.05). All nuclear morphometric parameters showed insignificant difference between low-grade and high-grade dysplasia (P<0.05). In cases of adenocarcinoma, nuclear area showed significant increase with increasing histologic grade (P>0.05), as shown in Fig. 2 (Table 3).

#### Cell cycle analysis

Table 4 showed that the examined cells in the gap 1 (G1) phase significantly decrease from control cases, low grade dysplasia, high grade dysplasia to carcinoma cases with higher grades (P>0.05). The percentage of proliferating cells increased significantly in adenomas than control. However, carcinomas showed decreased number of proliferating cells than adenomas. As regard

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	Low >25% [n (%)]	Moderate 25–50% [n (%)]	High <50% [ <i>n</i> (%)]	Total
Adenomas				
Low-grade dysplasia	1 (11.1)	2 (22.2)	6 (66.7)	9
High-grade dysplasia	1 (12.5)	2 (25)	5 (62.5)	8
Total	2 (10)	4 (25)	11 (65)	17
Adenocarcinoma*				
Grade I	4 (57.2)	2 (28.5)	1 (14.3)	7
Grade II	4 (66.6)	1 (16.7)	1 (16.7)	6
Grade III	3 (100)	0	0	3
Total	11 (68.9)	3 (18.8)	2 (12.3)	16

\*Significant difference at P value less than 0.05 using the  $\chi^2$  test.

#### Figure 2



Nuclear morphometry: (a) normal colonic mucosa, (b) adenoma with dysplasia, (c) grade I adenocarcinoma, and (d) grade III adenocarcinoma (hematoxylin and eosin, ×400).

cells in S phase and gap 2 (G2), there was insignificant difference between the studied groups (P<0.05). There was a significant increase of the percent of aneuploid cells between studied groups where they increase from low grade dysplasia to high grade dysplasia to carcinoma cases with increasing grades (P>0.05) (Fig. 3).

## Correlation between Bcl-2 expression and nuclear parameters

There was nonsignificant correlation between nuclear area and proportion of bcl-2 positive cells in cases of adenomas with low-grade and high-grade dysplasia. In carcinoma cases, there was a significant converse correlation between nuclear area and proportion of bcl-2 positive cells with different histologic grades.

Table 3 Nuclear I	morphometric	parameters of	the studied	cases
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	Area (µm <sup>2</sup> )	Diameter (µm)	Perimeter (µm)	Roundness	Texture	Mean grey
Normal colonic mucosa	12.9±1.5 <sup>a</sup>	5.1±0.2 <sup>a</sup>	14.3±0.7 <sup>a</sup>	1.23±0.06 <sup>a</sup>	48.1±7.1 <sup>a</sup>	234.8±1.2 <sup>a</sup>
Adenomas						
Low-grade dysplasia	18.5±2.2 <sup>b</sup>	6.0±0.4 <sup>a</sup>	17.0±1.2 <sup>a</sup>	1.20±0.02 <sup>a</sup>	118.2±21.7 <sup>a</sup>	219.0±6.2 <sup>a</sup>
High-grade dysplasia	20.4±1.3 <sup>b</sup>	6.4±0.1 <sup>a</sup>	17.9±0.5 <sup>a</sup>	1.20±0.02 <sup>a</sup>	69.6±30.9 <sup>a</sup>	231.5±7.9 <sup>a</sup>
Adenocarcinoma						
Grade I	23.7±2.1 <sup>c</sup>	6.8±0.4 <sup>a</sup>	19.2±0.9 <sup>a</sup>	1.19±0.01 <sup>a</sup>	62.8±19.4 <sup>a</sup>	233.6±6.1 <sup>a</sup>
Grade II	29.9±0.7 <sup>d</sup>	7.5±0.0 <sup>a</sup>	21.4±0.2 <sup>a</sup>	1.18±0.03 <sup>a</sup>	62.7±22.2 <sup>a</sup>	232.5±8.9 <sup>a</sup>
Grade III	35.2±3.2 <sup>e</sup>	8.2±0.5 <sup>a</sup>	23.2±1.3 <sup>a</sup>	1.17±0.005 <sup>a</sup>	101.1±13.6 <sup>a</sup>	230.4±6.8 <sup>a</sup>

All data are presented as mean±SD. All data with different super script letters (a, b, c, d, e) are significant at *P* value less than 0.05 using the analysis of variance test.

#### Table 4 Cell cycle indices of the studied groups

	G1cell %	S cell %	G2 cell %	Aneuploid cell %
Normal colonic mucosa	72.0	22.0	6.0	0.0
Adenomas*				
Low-grade dysplasia	35.8	36.8	18.4	9.0
High-grade dysplasia	19.0	42.5	22.5	16.0
Adenocarcinoma*				
Grade I	7.0	29.7	32.0	31.3
Grade II	1.7	14.3	29.7	54.3
Grade III	1.0	8.4	18.3	72.3

\*Significant difference at P value less than 0.05 using the  $\chi^2$  test.

#### Figure 3



Histogram of cell cycle indices: (a) normal colonic mucosa, (b) adenoma with dysplasia, (c) grade I adenocarcinoma, and (d) grade III adenocarcinoma.

In adenoma cases both of proliferating cells % (S+G2) and proportion of bcl-2 positive cells increased with significant correlation in low-grade and highgrade dysplasia. In carcinoma cases both parameters decreased with significant correlation in different histologic grades. As regard aneuploidy cells %, there was a significant inverse correlation with proportion of bcl-2 positive cells in dysplastic adenoma and carcinoma cases with different histologic grades (Table 5).

#### Discussion

Bcl-2 gene is a 26-kD protein that inhibits apoptosis and may lead to accumulation and propagation of cells having genetic mutation [8]. Apoptosis is a form of programmed cell death that happens in normal tissue development and regeneration. It can be activated by stress signals such as nutrient deprivation, reactive oxygen species and excessive mitogenic signaling associated with tumor initiation [24].

Increased proliferation which occurs due to oncogenic mutations is promoted by alterations in apoptotic pathways that finally lead to tumor growth. Colon apoptosis has a crucial role in intestinal turnover, so that the disruption of balance between apoptosis and proliferation facilitate tumor development and progression [25].

On the other hand, nuclear morphometry and the study of cell cycle by image cytometric analysis are very useful in understanding and predicting tumor development. Dysregulation of the cell cycle is the primary step in carcinogenesis and have an important role in tumor invasion and metastases [21].

This study aimed to evaluate immunohistochemical expression and image analysis of nuclear parameters in premalignant and malignant colon tumors.

In our study, normal mucosa of colon showed bcl-2 positive staining in the lower half of colonic crypts and surface epithelium were negatively stained for bcl-2. These results were also shown by previous studies [26,27]. They demonstrated that bcl-2 is normally expressed in the basal portion of colonic crypts which correspond to the progenitor cells. These cells are known to protect the regenerative compartment from cell death.

In the present study, 85% of the colon dysplasia (low and high grades) is positive for bcl-2 expression, however, its expression showed nonsignificant

#### Table 5 Correlation between bcl2 expression and nuclear parameters

	Proportion of bcl-2 positively stained cells				
	Low	Moderate	High		
	>25%	25–50%	<50%		
Nuclear area					
Adenomas					
Low-grade dysplasia	20.4	19.2	16		
High-grade dysplasia	21.9	19.7	19.5		
Adenocarcinoma*					
Grade I	25.6	24.1	21.3		
Grade II	30.6	30	29.1		
Grade III	37.6	36.4	31.5		
Aneuploid cells					
Adenomas*					
Low-grade dysplasia	11	11	5		
High-grade dysplasia	23	14	13		
Adenocarcinoma*					
Grade I	37	31	26		
Grade II	61	51	51		
Grade III	81	69	67		
Proliferating cell					
Adenomas*					
Low-grade dysplasia	36	64	65.5		
High-grade dysplasia	55	69	69		
Adenocarcinoma*					
Grade I	58	60	67		
Grade II	38	47	47		
Grade III	19	29	32		

\*Significant difference at *P* value less than 0.05 using the  $\chi^2$  test.

correlation with the degree of dysplasia. These results were in accordance with Elsharkawy [22] that showed 61% of cases of colon dysplasia showed bcl-2 immunopositivity with nonsignificant relation with the degree of dysplasia. On the other hand, 65% of colon dysplasia in our study showed high percentage of bcl-2 immunoreactivity (>50%) staining, while 25% had moderate staining (25–50%), and the remaining 15% showed low percentage of bcl-2 staining (<25%) with nonsignificant correlation between percentage of bcl-2 positivity and degree of dysplasia.

The prognostic significance of bcl-2 expression is controversial in colon cancer. While some reports showed a favorable prognostic role of bcl-2 [16,28], others demonstrated that bcl-2 is a poor prognostic marker for malignant tumors including colon cancer [29].

In the current study, bcl-2 positivity was detected in about 53% of CRC with a significant difference between its expression in carcinoma cases and cases of dysplastic adenoma. The percentage of positive cells decrease from low to high grade with inverse correlation with tumor differentiation. These results were in agreement with the study of Patil et al. [30] which showed that well-differentiated cases had immunoreactivity greater bcl-2 than poorly differentiated cases. However, a previous study bcl-2 overexpression demonstrated that was associated with high histological grades, resulting in aggressive tumor [29]. Other study did not detect any correlation between bcl-2 expression and histopathological grade [31]. This study showed that the percentage of bcl-2 positivity in grade III is significantly lower than that in grade I and grade II. Similar results were demonstrated by previous studies which showed increased of expression bcl-2 in adenomas than carcinomas indicating its role in the early stages of carcinogenesis [27,32]. Moreover, lack of bcl-2 expression has been associated with invasion, metastases, and recurrence of CRC [28].

The consonance between the antiapoptotic and proapoptotic bcl-2 family proteins is responsible for cell's fate. Studies have been performed to identify bcl-2 inhibitors, and to evaluate the clinical value of using bcl-2 antiapoptotic members as therapeutic targets for many malignant tumors [33].

Various methods can be used for analysis of cell cycle; however, in the present study we used image analysis for nuclear morphometry and cell cycle progression in colon adenoma and carcinoma. Morphometric analysis comprises measuring and counting to obtain accurate data for cell and tissue components that are important for diagnosis and prognosis of several tumors. Quantitative assessment of nuclear morphometry with computerized image analysis was done in many studies and correlated with prognostic factors of various malignancies including colon cancer, breast cancer, and renal cell carcinoma [34–36].

In the present study, all nuclear parameters showed nonsignificant increase in values with increasing dysplastic changes in cases of adenomas, while in colon carcinoma only nuclear area showed significant increase with increasing histologic grade (P<0.01). On the other hand, nuclear area showed statistically significant difference (P<0.01) between adenoma with high-grade dysplasia and grade I colon adenocarcinoma. This is in accordance with Yassen *et al.* [37] who suggested that nuclear area has a considerable role in prediction of carcinoma. They added that nuclear morphometry has also an important role in screening of patients with colon cancer who are at risk of metastases or recurrence after curative surgery.

Nuclear morphometric studies were verified in previous studies of various malignancies [38-40]. Mendacolli et al. [41] detected significant changes in nuclear morphometric and chromatin texture pattern between basal cell carcinoma and normal basal epithelium. Other studies showed a significant difference in nuclear morphometric parameters including perimeter, circularity, and major and minor axes ratio between hepatocellular carcinoma and adjacent tissue hepatocytes with high sensitivity and specificity for discrimination between neoplastic nonneoplastic hepatocytes [42,43]. and They determined that the incorporation of technological digital histological analysis for nuclear morphometry can help the pathologists in quantifying parameters that may not be detected by a subjective analysis.

The cell cycle is divided into four phases; the length of these cell cycle phases is an important characteristic of cell life [19]. The cell progression through these phases is controlled by endogenous and exogenous factors. On the other hand, the relationship between cell division and cell death represents a link between cell cycles and cell death programs that is present in all cells. Cancer is characterized by aberrant regulation, leading to uncontrolled proliferation and replicative immorality [44].

In our study of cell cycle by image cytometry, the examined cells in G1 phase showed significant

decrease (P < 0.01) from control cases, low-grade high-grade dysplasia dysplasia, to cases of carcinomas with increasing grades. In adenomas there is a significant increase in the percentage of euploid proliferating cells than in normal colonic mucosa. However, in carcinomas, the percentage of euploid proliferating cells decreases with advanced tumor grade, this was shown as large percent of cell population shift to the aneuploid category outside the normal cell cycle. Both S phase and G2 values increase significantly in carcinomas.

Previous flow cytometric study of DNA [45] in CRC suggested that DNA content of tumor cells is important for prognosis. The flow cytometric results showed that high S-phase values indicate that a large proportion of cells are proliferating, resulting in rapid growth of tumor. The study also demonstrated that DNA content, S-phase fraction, and tumor ploidy determination reveal important information about the biological behavior of colon cancer.

In accordance with our study, Elsharkawy [22] observed that DNA aneuploidy was more frequently detected in CRC than adenomas with statistically significant difference. They also demonstrated that there was a significant correlation between aneuploidy and degree of dysplasia in adenomas and increasing histological grades of carcinoma, suggesting the role of aneuploidy in predicting the malignant potential of adenoma.

Other studies showed that S-phase values also have a prognostic role in tumors of lymphatic system and breast and may be useful in predicting response to treatment in head and neck malignancies [46,47].

Cell death initiated by cell cycle checkpoint proceeds through apoptosis. Apoptosis is the form of cell death during development. It is clear that, the link between cell death and proliferation, is between apoptosis and the cell cycle [48]. In the present study, the percentage of proliferating cells (S+G2 phases) increased and showed significant correlation with the proportion of bcl-2 positive cells in cases of colon adenoma (lowgrade and high-grade dysplasia). On the contrary both parameters decrease in cases of colon carcinoma with significant correlation between the percentage of aneuploid cells and proportion of bcl-2 positive cells. These results point toward the role of altered expression of bcl-2 that occurs in the sequence of molecular events of cell cycle leading to colon carcinoma. Therefore, many preclinical studies supported the clinical potential of bcl-2 inhibitors as

single factor or in combination with other standard therapies to improve treatment responses and patient survival [33,49–52].

Finally, early diagnosis and prediction of patients at high risk for developing CRC is important. Therefore, data from computerized morphometry and cell cycle analysis together with immunohistochemistry have a considerable role in detecting the ability of high dysplastic colon cells to be turned into malignant cells.

#### Conclusion

Bcl-2 protein immunoreactivity play an important role in early event of colorectal carcinogenesis and its inhibitor may have a potential use in management of colon cancer. The acceptable reliability of immunohistochemical, nuclear area, and cell cycle analysis may serve as diagnostic and prognostic indicators in benign and malignant colorectal lesions.

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#### **Conflicts of interest**

There are no conflicts of interest.

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## Relation between 25-hydroxy vitamin D and diabetic retinopathy in Egyptian patients with type 2 diabetes mellitus

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#### Background/aim

Diabetic retinopathy (DR) is one of the leading causes of blindness in people whose ages range from 20 to 64 worldwide. This study aims to evaluate the association of serum 25-hydroxy (OH) vitamin D deficiency and DR in type 2 diabetes mellitus (T2DM) patients.

#### Patients and methods

A cross-sectional study was conducted on 90 Egyptian patients with T2DM. Patients were divided into two groups: group I included 44 patients without retinopathy and group II included 46 with retinopathy. Group II was subdivided into two subgroups, group IIa, which included 26 nonproliferative DR patients and group IIb, which included 20 proliferative DR patients. Anthropometric data and laboratory investigations, including fasting and the postprandial blood sugar, glycated hemoglobin, lipid profiles, and serum vitamin D level. A fundus examination was also performed.

#### Results

The present results exhibited a significant decrease (P<0.05) of serum vitamin D (25-OH-vitamin D) in patients with T2DM and this deficiency is more in group II in comparison to group I and in a patient with proliferative DR (group IIb) compared to nonproliferative DR (group IIa). Also, there was a downward trend in serum vitamin D with the severity of DR. Also 25-OH-vitamin D level was inversely correlated with fasting, postprandial blood sugar, and glycated hemoglobin. A cut-off value for serum vitamin D levels of less than or equal to 12.03 ng/ml served as a sensitive indicator for DR.

#### Conclusion

25-OH-vitamin D deficiency is highly prevalent in Egyptian patients with T2DM and this deficiency is highly associated with the presence and severity of DR.

#### Keywords:

25-hydroxy-vitamin D, diabetic retinopathy, nonproliferative, proliferative

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#### Introduction

The type 2 diabetes mellitus (T2DM); one of main threats to aging population health in the 21<sup>st</sup> century, is described as a global epidemic since it affects health and economy of almost all the countries irrespective of their socioeconomic status or their geographic location [1].

Diabetic retinopathy (DR) is one of the leading causes of blindness in people 20–64 years of age [2]. It is believed that nearly all the patients with type 1 and 60% of the type 2 diabetes will have evidence of DR on examination 20 years after the onset of diabetes [3]. DR, is characterized as a neurovascular disease entity that results from hyperglycemia-induced changes in blood-retinal barrier and the retinal vasculature [4].

Many vasoactive factors are evoked by hyperglycemia and retinal hypoxia. These factors induce pathology in a variety of cell types, including glia, neurons, and microvasculature. A primary factor in the regulation of vessel patency throughout the body and retina is vascular endothelial growth factor (VEGF). Early in diabetes, the VEGF system is disturbed, and there is an interaction between it and other vasoactive factors, which stimulate the breakdown of blood-retinal barrier and causes angiogenesis leading to proliferative diabetic retinopathy (PDR) [5].

Insufficiency of vitamin D is very prevalent worldwide. 25-hydroxy-vitamin D3 (abbreviated as 25OH-D3) is a form of vitamin D, produced in the liver by hydroxylation of vitamin D3 (cholecalciferol) by the enzyme vitamin-D 25-hydroxylase. Different epidemiological studies have shown that more than

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40% of adult populations are at risk of inadequate 25-OH vitamin D. Moreover, strong correlations exist between 25-OH vitamin D status, obesity, and T2DM. However, it was found that 25-OH vitamin D deficiency is predisposing factor for diabetes development and increasing hearing loss [6]. Animal studies have suggested that 25-OH vitamin D active metabolite supplementation protects against retinal neo-vascularization and other studies have documented the anti-angiogenic effects of 25-OH vitamin D primarily in the tumor models [7].

Many previous studies addressed the potential role of 25-OH vitamin D in the pathogenesis of DR [8–10]. To our knowledge, in Egypt, there is data about the association of diabetic peripheral neuropathy and the 25-OH vitamin D deficiency in T2DM [11] and T1DM in children [12]. Our study aimed to assess the association between DR and 25-OH vitamin D status in T2DM in a sample of Egyptian patients and the relation between 25-OH vitamin D deficiencies and the severity of retinopathy.

#### Patients and methods Patients

This cros- sectional study included 90 Egyptian participants, aged between 31 and 65 years from April 2019 to March 2020. Patients were recruited from the Internal Medicine, Endocrinology, and Ophthalmology Departments of Al-Zahraa University Hospital. This study was done in accordance with the Declaration of the Helsinki guidelines. A written informed consent was taken from all the participants after a full explanation. Approval of the Ethics Board of Al Azhar University was obtained.

The included participants were divided into two groups as follows:

Group I consisted of 44 patients without DR.

Group II included 46 patients with DR. According to the Early Treatment Diabetic Retinopathy Study [13], this group was further divided into two subgroups, group IIa: 26 nonproliferative diabetic retinopathy patients (NPDR) and group IIb: 20 PDR patients.

#### Inclusion criteria

Adult patients aged 31–65 years diagnosed as having T2DM based on the American Diabetes Association criteria were included in this study.

#### **Exclusion criteria**

The exclusion criteria including T1DM, patients with history of hypertension, cardiovascular diseases, chronic kidney or liver disease, cancer, hypoparathyroidism, or hyperparathyroidism. Patients on 25-OH vitamin D replacement therapy, antioxidants,or medications affecting 25-OH vitamin-D metabolisms, such as phenobarbital, phenytoin, and rifampin were excluded.

Ocular exclusion criteria: dense media opacities, history of uveitis, any systemic diseases or medications affecting the retina, exposure to ocular trauma or surgery. Patients who had any posterior segment pathology except DR were also excluded.

#### Examination protocol and study measurements

The following examinations were conducted on all patients:

Full medical history, including duration and treatment of diabetes, complete physical examination, calculation of BMI according to the formula weight divided by the square of the height (kg/m<sup>2</sup>) [14]. In addition to, complete ophthalmic examination including the uncorrected and the best-corrected visual acuity using Landolt's ring chart, refraction, and examination of slit lamp. Intraocular pressure was measured using Goldman applanation tonometer. A dilated fundus examination for DR grading was performed with the aid of +90 D noncontact lens.

#### Laboratory investigations

The laboratory investigations, which included fasting blood sugar (FBS) and postprandial blood glucose levels, total cholesterol, triglycerides, a high-density lipoprotein cholesterol and a low-density lipoprotein cholesterol were carried out using an automated chemistry analyzer (Cobas c311; Roche, Sandhofer Strasse 116 68305 Mannheim, Germany), according to manufacturer's instructions. Glycated hemoglobin (HbA1c) measurement was performed using the immunoassay turbidimetric inhibition method, according to the manufacture instruction of dimension x-pand plus supplied from Siemens (Siemens Healthcare Diagnostics Inc., Newark, Delaware, USA). Serum calcium and phosphorus were measured by a biochemical automatic analyser (Cobas c702; Roche, Shanghai, China).

Determination of serum 25-OH vitamin D was performed using the enzyme immunoassay method using the kit supplied from MicroVue (Quidel Corporation, San Diego, California, USA). Interpretation of the test: deficient: less than 20, insufficient: 20–29, while sufficient: 30–100 ng/ml, according to the method of Holick [15].

#### Statistical analysis

Data were collected, revised, coded, and entered into Statistical Package for the Social Science (IBM SPSS, Chicago, Illinois, USA), version 15. Quantitative data were presented as the mean±SD. Comparision of the two groups was done using independent t test, while comparison between more than two independent groups with the parametric distribution was conducted by using the one-eay analysis of variance. Qualitative variables were presented as the number and the percentages. Comparing the groups regarding the qualitative data was conducted using the  $\chi^2$  test. The correlation between different studied parameters was done using Pearson's correlation coefficient. A *P* value less than 0.05 is considered significant. Receiver operating characteristic curve was used for prediction of DR.

#### Results

#### Demographic data of studied patients

The present study included 35 (39%) males and 55 (61%) females of Egyptian patients with T2DM. Their ages ranged from 31 to 65 years. All patients underwent fundus examination and according to the findings, our patients were divided into two groups: group I:

included 44 diabetic patients without retinopathy and group II that included 46 diabetic patient with DR. The demographic and laboratory data of both groups are shown in Table 1. By comparing group I and group II there was an insignificant difference as regard age and sex while disease duration significantly increase (P<0.05) in group II in comparison to group I, but BMI was significantly low (P<0.05) in the group II compared to the group I (Table 1).

Moreover, there was significant increase (P<0.05) in fasting, postprandial blood sugar, and HbA1c in group II (221±80, 291±85 mg/dl, and 9.96±2.07%, respectively) when compared to group I (182±76, 238±88 mg/dl, and 8.74±2.09%, respectively), while, there was significant decrease (P<0.05) of serum 25-OH vitamin D and very low-density lipoprotein in group II (11.66±1.60 ng/ml and 28.79±6.68 mg/dl), when compared to group I (15.52±9.94 ng/ml and 44.62±21.80 mg/dl). Also, there was an insignificant difference between the two groups as regards other parameters (Table 1).

As regard grading of retinopathy, our patients with DR in group II were divided into two groups, group IIa, which includes 26 NPDR patients, and group IIb, which involved 20 PDR patients.

Table 1 Comparison between the diabetic group without retinopathy (group I) and the diabetic group with retinopathy (group II) as regards demographic and biochemical data

	Group I (diabetic without retinopathy)	Group II (diabetic with retinopathy)	
Parameters	N=44	N=46	P value
Age (years)	48.68±6.74	52.26±7.75	0.101
Sex [n (%)]			
Male	22 (50)	15 (32.6)	0.094**
Female	22 (50)	31 (67.4)	
Disease duration (years)	8.38±6.28	15.35±6.912	0.000*
Weight (kg)	96.01±18 .94	86.11±16.44	0.000*
Height (cm)	165.18±8.07	163.59±8.34	0.359
BMI (kg/m <sup>2</sup> )	35.06±5.43	31.59±5.30	0.003*
FBS (mg/dl)	182±76	221±80	0.000*
PBS (mg/dl)	238±88	291±85	0.005*
HbA1c (%)	9.85±2.09	9.96±2.07	0.007*
Calcium (mg/dl)	9.06±0.52	9.01±0.59	0.642
Phosphorus (mg/dl)	4.53±0.47	4.56±0.79	0.833
Albumin (gm/dl)	4.11±0.30	4.20±0.45	0.574
Cholesterol (mg/dl)	188.39±42.97	199.20±42.38	0.233
Triglyceride (mg/dl)	189.34±90.45	179.22±72.06	0.558
HDL (mg/dl)	39.23±8. 24	41.36±8.77	0.239
LDL (mg/dl)	111.31±43.89	119.62±37.85	0.282
VLDL (mg/dl)	44.62±20.80	28.79±6.68	0.006*
25-OH vitamin D (ng/ml)	15.52±9.94	11.66±1.60	0.011*
Oral treated [n (%)]	11 (25)	20 (43.5)	0.053**
Insulin treated [n (%)]	33 (75)	26 (56.5)	

FBS, fasting blood sugar; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PBS, postprandial blood sugar; VLDL, very low-density lipoprotein. \*Significant difference at *P* value less than 0.05, using independent *t* test. \*\*Insignificant difference at *P* more than 0.05, using  $\chi^2$  test.

On comparing between the two subgroups, as regard demographic and biochemical data, where there was significant increase (P<0.05) in disease duration in group IIb in comparison to group IIa, also there was significant increase (P<0.05) in fasting, postprandial blood sugar and HbA1c in group IIb (225.75±89.73, 307.95±97.87 mg/dl, and 10.11±2.01%, respectively) in comparison to group IIa (217.08±72.26, 277.58 ±72.56 mg/dl and 9.85±2.16% respectively). Also, 25-OH vitamin D level was significantly lower (P<0.05) in group IIb (11.06±1.59) when compared with group IIa (12.13±1.47), as shown in Table 2.

Also, there is an insignificant difference (P>0.05) between males and females regarding weight, BMI, and 25-OH vitamin D level, as shown in Table 3.

As regard serum 25-OH vitamin D was within the normal level of four (4.4%) patients, 76 (84.4%) patients had insufficient level, while 10 (11.2%) patients had deficient level. There was a significant decrease in vitamin D level in group IIb (11.06±1.59) when compared to group IIa and group I (12.13±1.47, 15.52±9.94, respectively) and post-hoc test revealed a highly significant difference between group I and group

Table 2 Comparison between nonproliferative diabetic retinopathy (group IIa) and proliferative diabetic retinopathy (group IIb) groups as regards demographic and biochemical data

Parameters	Group IIa (NPDR) (N=26)	Group IIb (PDR) (N=20)	P value
Age (years)	56.96±8.60	55.35±7.79	0.000*
Sex [ <i>n</i> (%)]			
Male	5 (19.2)	10 (50.0)	0.020**
Female	21 (80.8)	10 (50.0)	
Disease duration (years)	13.42±6.40	19.35±11.44	0.000*
Weight (kg)	86.88±14.94	85.10±18.56	0.033*
Height (cm)	163.15±7.97	164.15±8.98	0.607
BMI (kg/m <sup>2</sup> )	32.29±5.97	30.69±4.26	0.007*
FBS (mg/dl)	217.08±72.26	225.75±89.73	0.032*
PBS (mg/dl)	277.58±72.56	307.95±97.87	0.010*
HbA1c (%)	9.85±2.16	10.11±2.01	0.024*
Calcium (mg/dl)	9.10±0.60	8.89±0.58	0.377
Phosphorus (mg/dl)	4.35±0.87	4.84±0.59	0.038*
Serum albumin (g/dl)	4.08±0.48	4.32±0.43	0.520
Cholesterol (mg/dl)	211.81±44.72	182.80±33.49	0.034*
Triglyceride (mg/dl)	186.81±74.01	169.35±70.07	0.652
HDL (mg/dl)	41.95±8.82	40.59±8.88	0.435
LDL (mg/dl)	128.31±43.21	108.33±26.44	0.101
VLDL (mg/dl)	29.10±6.71	28.44±7.05	0.026*
Vitamin D (ng/ml)	12.13±1.47	11.0 6±1.59	0.035*

FBS, fasting blood sugar; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NPDR, nonproliferative diabetic retinopathy; PBS, postprandial blood sugar; PDR, proliferative diabetic retinopathy; VLDL, very low-density lipoprotein. \*Significant difference at *P* value less than 0.05, using independent *t* test. \*\*Insignificant difference at *P* more than 0.05, using  $\chi^2$  test.

Table 3 Comparison between males and females in all patients with diabetic retinopathy (group II), nonproliferative diabetic retinopathy (group IIa) and proliferative diabetic retinopathy (group IIb) groups regarding weight, BMI, and 25-hydroxy vitamin D level

	Group II: total diabetic with retinopathy			Group IIa: NPDR			Group IIb: PDR		
_	Female	Male	P value	Female	Male	P value	Female	Male	P value
Weight	83.23±14.35	92.07±19.26	0.087*	85.62±14.39	92.20±17.80	0.387*	78.20±13.62	92.00±20.89	0.097*
BMI	31.75±5.70	31.28±4.52	0.784*	32.40 ±6.15	31.84±5.75	0.856*	30.38±4.61	31.00±4.10	0.754*
Vit D	11.65±1.71	11.69±1.40	0.945*	12.02±1.59	12.60±0.72	0.440*	10.89±1.78	11.23±1.46	0.638*
Normal [ <i>n</i> (%)]	0	0	0.459**	0	0	0.369**	0	0	0.329**
Insufficient [n (%)]	24 (77.4)	13 (86.7)		18 (85.7)	5 (100.0)		6 (60.0)	8 (80.0)	
Deficient [n (%)]	7 (22.6)	2 (13.3)		3 (14.3)	0		4 (40.0)	2 (20.0)	

NPDR, nonproliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy. Insignificant difference at *P* value more than 0.05 using independent *t* test\* or  $\chi^2$  test\*\*.

Table 4 Comparison of serum 25-hydroxy vitamin D level in the diabetic group without retinopathy (group I), nonproliferati	ve
diabetic retinopathy (group IIa), and proliferative diabetic retinopathy (group IIb) groups	

	Group I (diabetic without retinopathy)	Group IIa (NPDR)	Group IIb (PDR)	
Vitamin D	N=44	N=26	<i>N</i> =20	P value
Mean±SD	15.52±9.94	12.13±1.47	11.06±1.59	
Range	9.93-63.2	8.59–15.4	8.59-13.8	0.035*
Normal [n (%)]	4 (9.1)	0	0	0.006*
Insufficient [n (%)]	39 (88.6)	23 (88.5)	14 (70.0)	
Deficient [n (%)]	1 (2.3)	3 (11.5)	6 (30.0)	
Post-hoc analysis				
Parameters	P <sub>1</sub>	P <sub>2</sub>	P <sub>3</sub>	
Quantitative vitamin D	0.056	0.022*	0.612	
Qualitative vitamin D	0.089	0.002*	0.294	

NPDR, nonproliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy. \*Significant difference at *P* value less than 0.05, using one-way analysis of variance test with post-hoc analysis using LSD. *P*1: comparison between diabetic without retinopathy (group I) and NPDR group (group IIa). *P*2: comparison between diabetic without retinopathy (group I) and PDR group (group IIb). *P*3: comparison between NPDR (group IIa) and PDR (group IIb) groups.

IIa, the distribution of number of patients in each group with respect to level of 25-OH vitamin D are shown in Table 4.

Pearson's correlation of 25-OH vitamin D with the studied parameters in group II (diabetic patients with retinopathy) showed a significant inverse correlation between it and each of fasting, postprandial blood sugar, and HbA1c (Fig. 1). Also a significant negative correlation between 25-OH vitamin D and serum calcium was noted in group II.

Receiver operating characteristic curve of 25-OH vitamin D level was conducted for the prediction of DR in diabetic patients. At serum concentration of 25-OH vitamin D level best cut off value is less than or

Figure 1

equal to 12.03, sensitivity was 65.22%, specificity was 68.18%, the positive predictive value was 68.2%, and the negative predictive value was 65.2%.

Multivariate regression analysis using the backward: Wald method shows that age more than 49 and disease duration more than 9 years was found to be significantly associated to retinopathy with P value of 0.004 and 0.006, respectively, and with odds ration (OR) [95% confidence interval (CI)] of 7.945 (1.964 - 32.140)and 5.566 (1.619 - 19.135).respectively, followed by FBS more than 205 and BMI less than or equal to 34.3 with P value of and 0.048, respectively, and with OR 0.017 (95% CI) of 4.588 (1.307-16.103) and 3.335 (1.011-10.997), respectively; while 25-OH vitamin



Pearson's correlation between HbA1c and 25-hydroxy vitamin D in diabetic patients with retinopathy (group II). HbA1c, glycated hemoglobin.

						95% C	95% CI for OR	
Parameters	В	SE	Wald	P value	OR	Lower	Upper	
Vitamin D<12.03	0.996	0.600	2.754	0.097	2.708	0.835	8.781	
BMI≤34.3	1.204	0.609	3.913	0.048*	3.335	1.011	10.997	
DD>9	1.717	0.630	7.426	0.006*	5.566	1.619	19.135	
Age >49	2.073	0.713	8.449	0.004*	7.945	1.964	32.140	
FBS>205	1.523	0.641	5.654	0.017*	4.588	1.307	16.103	
Constant	-4.218	0.946	19.886	0.000*	0.015			

Table 5 Final model of multivariate logistic regression analysis using backward Wald method for the association between retinopathy and the other parameters

Variable(s) entered on step 1: vitamin D, weight, BMI, disease duration, age, fasting blood sugar, and postprandial blood glucose. CI, confidence interval; OR, odds ratio. \*Significant difference at *P* value less than 0.05.

Figure 2



Receiver operating characteristic (ROC) curve of 25-hydroxy vitamin D in diabetic patients with retinopathy (group II). The cut-off value is less than or equal to 12.03, sensitivity was 65.22%, specificity was 68.18%, positive predictive value was 68.2%, and negative predictive value was 65.2%.

D not found as an independent factor for retinopathy with P value of 0.097 and OR (95% CI) of 2.708 (0.835–8.781), as shown in Table 5, Fig. 2.

#### Discussion

In our study, there was a statistically significant decrease of serum 25-OH vitamin D in most patients with type 2 diabetes. This decrease was more in diabetic patients with retinopathy (group II) in comparison to patients without retinopathy (group I) and in patients with PDR (IIb) compared with NPDR (IIa). Also, there was a downward trend in serum 25-OH vitamin D with the severity of DR.

The relation between vitamin D deficiency and T2DM was reported more than 30 years ago. In 1988,

Pietschmann *et al.* [16] found that serum vitamin D was lower in diabetic patients than nondiabetic control. Other studies concluded that serum vitamin D was low in T2DM and this is one of the contributing factors for the development of T2DM [17,18].

This agrees with the performed study by Dave et al. [19] on 98 patients with T2DM; all patients had vitamin D insufficient. Thirty-nine patients had no DR, 38 had mild to moderate NPDR, 15 had severe NPDR, and six patients had PDR. They found that 25-OH vitamin D decreased significantly in patients with PDR than those without retinopathy. In agreement with our study, Nadri et al. [20] found that serum 25-OH vitamin D was significantly low in T2DM patients and lowest in PDR patients. Their study was conducted on 72 patients with T2DM, 24 patients without retinopathy, 24 patients with NPDR, and 24 with PDR and vitamin D levels. Also consistent with our results Dinesh and Anil Kumar [21] in their study on 412 patients with T2DM, found that 7% had 25-OH vitamin D level more than 30 ng/ml, 13% had a level between 20 and 30 and 80% had less than 20 ng/ ml. They concluded that T2DM patients with retinopathy were found to have significant 25-OH vitamin D deficiency compared to those without retinopathy.

25-OH vitamin D has a suppressive function with antiangiogenic and anti-inflammatory effects in the pathogenesis of DR. Mantell *et al.* [22] showed that retinal new vascularization was inhibited by vitamin D in induced ischemic retinopathy mouse model. 25-OH vitamin D inhibits VEGF induced endothelial cell proliferation, elongation, and sprouting. Also, Albert *et al.* [7] proposed that 25-OH vitamin D interrupts the angiogenesis signalling pathway and induces endothelial cell apoptosis. Chronic inflammation leads to the degeneration of retinal pigment cells. However the anti-inflammatory effect of vitamin D exerts by inhibition of proliferation of natural killer cells, lymphocytes, and many pro-inflammatory cytokines. Also, metalloproteinase, MMP-9 production by inflammatory cells is inhibited by vitamin D [23].

Alcubierre *et al.* [24] reported the association of 25-OH vitamin D deficiency with the existence and extent of DR in T2DM in their study on two groups of patients. One humdred fourty-four without retinopathy and 139 patients with retinopathy, also patients with low concentration of vitamin D had advanced stage of DR.

In agreement with our findings, a Chinese study, which was conducted by He *et al.* [8] on 1520 patients with type 2 diabetes and was divided into three groups according to the results of their fundus oculi, where the percentage of patients with no DR were, 41.12%; non-PDR were 36.97%; and PDR were 21.91%, they concluded that patients with PDR 25-OH vitamin D was lower than other two groups, also there was a stepwise decrease in serum 25-OH vitamin D with the severity of DR.

Another research agrees with our findings [25] and found that patients with DR had significantly low serum vitamin D in comparison with those without retinopathy. They measured serum 25-OH vitamin D in 136 patients with T2DM and concluded that low serum 25-OH vitamin D level was an independent indicator of DR, diabetic neuropathy, and HbA1c in T2DM patients.

Also, our results are consistent with Suzuki *et al.* [26], who showed in their study on 581 Japanese diabetic patients and 51 normal participants that patients with microvascular complications had significantly low serum vitamin D when compared with patients without complications.

Another study agrees with our results, Gungor *et al.* [27], studied 50 early-stage DR patients with 25-OH vitamin D deficiency and 50 early-stage DR patients without 25-OH vitamin D deficiency. The same ophthalmologist examined all patients, and optical coherence tomography was used to determine the retinal nerve fiber thickness. Also 25-OH vitamin D levels were measured by using a radioimmunoassay. They reported that patients with 25-OH vitamin D and reduced mean of retinal nerve fiber thickness compared with patients without 25-OH vitamin D deficiency.

As regards the correlation of serum 25-OH vitamin D with studied parameters, it was found that there was a

significant negative correlation between 25-OH vitamin D and each of fasting, postprandial blood sugar, and HbA1c. Our results agree with Ghavam *et al.* [28], who found an inverse linear relationship between 25-OH vitamin D with HbA1c, FBS, BMI, and disease duration. Also, in agreement with our results, a study was done by Alkhatatbeh and Abdul-Razzak [29], where there were significant inverse correlations between the duration of DM and HbA1c and fasting blood glucose levels. Extended previous studies are in agreement with our results [17,25,30].

#### Conclusion

This cross-sectional study demonstrated that vitamin D deficiency is highly prevalent in type 2 diabetic patients, and this deficiency is highly associated with the presence and severity of DR. These findings highlight the role of 25-OH vitamin D in the pathogenesis of the DR.

#### Recommendation

Further studies on large sample sizes are recommended to assess the role 25-OH vitamin D deficiency in the development of the DR and other diabetic microvascular complications. Also, further follow up of the patients after vitamin D supplementation to study the effect on retinopathy.

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#### **Conflicts of interest**

There are no conflicts of interest.

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### Impact of adding 3% titanium oxide nanoparticles in different denture base materials on some of the biological, physical, and mechanical properties properties: *In-vitro* study Wessam M. Dehis, Ayman F. Elawady, Menatallah M. ElHotieby, Sherihan M. Eissa

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#### Background/aim

Acrylic resin denture base material with all its varieties and curing techniques enormously influence their physical, biological, and mechanical properties. This study designed to evaluate and compare the impact of acrylic resin reinforcement with titanium oxide nanoparticles (TiO<sub>2</sub>NP) on physical, biological, and mechanical criteria of two dissimilar denture base materials.

#### Materials and methods

This study was constructed on 120 specimens of 2 different types of acrylic resin denture base materials. The specimens were divided equally into 2 groups (60 each), group I (heat cured acrylic resin) and group II (microwave cured acrylic resin). Each group was additionally divided into two subgroups (each 30). Subgroup A as control, while subgroup B was modified with 3% TiO<sub>2</sub>NP. Biological evaluation was performed on *Enterococcus faecalis by using antimicrobial assay by agar diffusion test* color stability via employing spectrophotometer, and the Modulus of elasticity through universal testing machine.

#### Results

Regarding the results of biological assessment, groups IA and IIA (control groups) revealed no inhibitory zone, while groups modified with 3% TiO<sub>2</sub>NP group IIB demonstrated significantly higher (P < 0.05) inhibitory zone when compared with group IB. Concerning color stability, Regarding color stability, Group IA and IIA (control groups) demonstrated the highest color changes, while Group IB and IIB (modified groups) demonstrated the lowest color changes. In modulus of elasticity, groups IB and IIB were significantly higher (P < 0.05) than groups IIA and IIB. **Conclusion** 

Adding 3%  $TiO_2NP$  to heat and microwave cured resins induced antibacterial characteristics against E. faecalis. Besides improvement of the evaluated mechanical (modulus of elasticity) and color stability properties.

#### Keywords:

biological, denture base material, nanoparticles, physical, titanium oxide, mechanical properties

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Wessam M. Dehis was responsible for conceptualization, data curation, methodology, article administration, supervision, review, editing, and submission of the manuscript to the journal. Ayman F. Elawady helped in visualization, laboratory investigations, and reviewing. Menatallah M. Elhotaby was taking a part in data and laboratory investigations. Sherihan M. Eissa aided in data curation, methodology, formal analysis, validation, laboratory investigations, interpretations, and statistical analysis. All authors read and approved the final manuscript.

Previous publication/presentations mentioned: (a) A comparative clinical study of the effect of denture cleansing on the surface roughness and hardness of two denture base materials. (b) Mechanical properties, color stability and biological characteristics of acrylic resin denture base materials containing titanium oxide

nanoparticles: *in-vitro* study. (c) Dimensional accuracy of implant impression obtained from poly-siloxane condensation silicone: an *in-vitro* study. (e) Flexural and tensile strength of acrylic resin denture base materials processed by three different methods. (f) Comparative study clarifying the most suitable material to be used as partial denture clasps.

#### Introduction

Denture base is determined as the denture's foundation which substitutes the whole dentition and related structures in both maxilla and mandible through its

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wide-ranging values [1]. Resting on the fundamental areas, prosthetic teeth attachment, transferring the intense occlusal forces to the basal structures besides replacing both bulk and shape of the underlying alveolar tissues are examples of its importance [2]. Denture base material has two main forms; metallic (cobaltchromium alloy) and nonmetallic (acrylic resin) [3,4]. Biocompatibility and advancement of the nonmetallic denture base's properties justify its remarkable employment in dentistry than the metallic one [5].

Acrylic resin as the outmost utilized denture base form is supplied as both liquid and powder. The liquid (monomer) embraces of chiefly unpolymerized methyl meth acrylate (MMA), petty percent of hydroquinone as an inhibitor and glycol dimethacrylate as a cross linking agent enhancing its distortion resistance [6,7]. The acrylic resin's extra response that necessitates initiator stimulation through energy for processing to take place is known as polymerization reaction [8].

Heat cure acrylic resin is the outmost regularly nonmetallic employed denture base whose polymerization reaction relies on thermal energy to take place represented as either a short or long water bath cycle [9]. Although heat cure polymethyl meth acrylate (PMMA) possesses several notable advantages, including its nontoxic nature, insolubility, pleasing aesthetics, affordability, extended shelf life, and ease of processing and repair, it is also important to acknowledge its associated drawbacks. These drawbacks such tissue hypersensitivity, as instability, dimensional discoloration, and susceptibility to bacterial colonization. As a result, it necessitates the development of innovative resins and employs advanced processing techniques in order to enhance its overall properties and functions [10-12].

Anatase is the most favorite crystalline form of titanium oxide's  $(TiO_2)$  assembly, owing to its nontoxicity, great surface area, enormous photo catalytic activity, reasonably affordable besides both its photo chemical and minimal temperature Thus, anatase- $TiO_2$  is employed for stability. disinfection and purification of different environmental elements as water and air [13,14]. Evolution in PMMA characteristics via addition of formulated titanium oxide nanoparticle (TiO<sub>2</sub>NP) has been stated recently and apparent in acrylic resin's physical, biological, mechanical, and viscoelastic criteria advancement. Hence, abundant utilization of TiO<sub>2</sub>NP in versatile dental specialties is currently apparent [10,15].

One of the recent acrylic resin form's innovations took place via microwave curing which is remarkably di-methacrylate distinguished via its liquid (monomer) instead of PMMA, together with attraction and rehabilitation of microwave energy polymerization employed for into evenlv disseminated dielectric heat which speedily elevates the temperature using the nonmetallic microwave translucent oven flask [16,17]. Sequentially, microwave cured acrylic resin denture base is advantageous with its dimensional stability, trivial residual monomer content, enhanced adaptation, nominal time, and properties advancement [18,19].

Because acrylic resin is porous, prosthetic patients are more susceptible to the formation of oral biofilm and intraoral bacterial infections. This susceptibility is exacerbated by oral hygiene practices and the presence of infectious disorders that promote bacterial adhesion and growth [20]. *Enterococcus faecalis (E. faecalis)*, a strong and highly prevalent intra-oral bacterial species, is particularly problematic in immunocompromised and prosthetic patients, potentially causing harm to these individuals. As a result, when selecting the base material for dentures, considering the biological factor becomes priority [21,22].

Since the acrylic resin denture base material with all its diversities is frequently exposed to liquid sorption, food debris, ecological conditions, and absorbing various contaminants ending up with discoloration and inferior esthetics. Hence, color stability is a crucial physical parameter in denture base material [23]. Methods employed for assessing color stability are either subjective or objective and the spectrophotometer with all its natures is rated as the most preferable objective technique for its easiness and accuracy [24].

Moreover, while denture base resins are often exposed to repeated mechanical stresses within the oral cavity, which can negatively affect their durability and functionality, only a limited number of studies have explored their mechanical characteristics under dynamic loads during activities like chewing and swallowing, as opposed to static loads. Permanent deformation caused by mastication can be tolerated by denture base materials possessing a higher modulus of elasticity. Because of flexure, upper denture fractures always happen through the midline of the denture. As a result, the denture foundation needs to have enough flexural modulus to withstand fracture [25].

Prosthetic appliances' durability and success greatly rely on their denture base resins' features whether biological, physical, or mechanical. These properties can be simply modified by incorporating any additive to PMMA [7]. As the existing literatures lacked comprehensive discussions regarding the influence of incorporating  $TiO_2NP$  into various types of acrylic resin denture base materials, this research was designed with the dual objective of assessing and contrasting the effects of reinforcing acrylic resin with  $TiO_2NP$  on the biological, physical, and mechanical properties of two distinct denture base materials.

#### Materials and methods Materials

This study was conducted using two distinct forms of accessible acrylic resin denture base materials, including the conventional heat cured acrylic resin (Acrostone; Acrostone Dental Factory—Industrial Zone, Salam City A.R. E-WHW Plastic, England) and Microwave cured acrylic resin (Protechno; Poligono Emporda Internacional, Garrotaxa, Vilamalla Girona, Spain). Moreover, TiO<sub>2</sub>NP (Sigma Aldrich company, USA) were added in modified groups.

#### Study design

A total number of 120 specimens were constructed utilizing metal patterns with different dimensions and shapes vary according to evaluated property and fabricated according to ADA specification no. 12. A metal disc of 10 mm in diameter ×2 mm in thickness was fabricated for biological property assessment, whereas a metal disc of 50 mm×0.05 mm in diameter and thickness for color stability evaluation, while a rectangular metal plate of 60 mm lengths × 10 mm width × 2 mm thicknesses for mechanical properties evaluation. These 120 specimens were divided equally into two main groups, group I of conventional heat cured acrylic resin and group II of microwave cured acrylic resin. Each group was additionally divided into two subgroups (30 each) as follows:

- (1) Group IA: Conventional heat cured PMMA control specimens.
- (2) Group IB: Specimens as group IA modified with 3% TiO<sub>2</sub>NP.
- (3) Group IIA: Microwave cured PMMA control specimens.
- (4) Group IIB: Specimens as group IIA modified with 3% TiO<sub>2</sub>NP.

The antibacterial activity against *E. faecalis* was assessed by measuring the diameter of the inhibition

zone in millimeters representing the biological properties. The color changes were assessed utilizing spectrophotometer to evaluate physical properties, and the modulus of elasticity was evaluated by universal testing machine to estimate the mechanical criteria.

#### Sample size calculation

Sample size was considered based on a preceding study as a reference [26]. Hence, the apparently passable sample size was 10/subgroup, once each group's response was ordinarily spread through standard deviation 0.11, difference 0.15, 80% power and 0.05 type I error probability. Independent *t*-test was performed by utilizing P.S. Power3.1.6.

#### Ethical approval

The current study adhered to the ethical guidelines outlined by the World Medical Association's Code of Ethics, following the principles set forth in the 1975 Declaration of Helsinki. Approval for this research was obtained from the Medical Research Ethical Committee (MREC) of the National Research Centre (NRC) in Cairo, Egypt, under approval number 54312012023.

#### Methods

Conventional metal flask was employed to attain molds of conventional heat cured acrylic resin while a special microwave plastic flask was purchased from Tecno-Flask (Protechno, Can Viloca, Spain) and utilized for the microwave one. The inferior section of the dental flask was occupied with dental plaster that was bought from Elite (Rock Stone, Zhermack Clinical, Italy), which was blended rendering to the manufacturer's guidelines (i.e., 50 ml/100 g). Each of the three metal patterns utilized in the current research was coated with the separating medium; that was purchased from Acrostone, Egypt, followed by another layer of plaster mix. Sequential to plaster setting (30 min), coating both the plaster and metal patterns with a separating medium then another layer of plaster was infused into the superior part of the flask with vibration by aid of mold vibrator. Plaster was left to harden for (60 min), then finally the flask was deflasked, metal patterns were detached, and the mold was gained which later on helped in construction of acrylic resin specimens.

#### Specimen preparation

The conventional heat cured PMMA was prepared and packed using a stainless-steel spatula following the manufacturer's guidelines. As the mixture approached the dough stage, it was carefully placed into the plaster mold. The metal flask was then compressed using a hydraulic press and positioned in the water bath curing unit (water bath curing unit; type 5518, KaVo EWL, Biberach, Germany) for a duration of 30 min at 70°C, accompanied by an extra 30 min at 100°C for the heat curing process. Afterward, the flask was grabbed out of the water bath and left till reaching room temperature before deflasking. Finally, finishing and polishing of the specimens were carried out.

The microwave-cured denture base material was prepared according to the manufacturer's instructions, which specified a powder-to-liquid ratio of 2:1 by weight (a powder ECO-CRYL M). Once the mixture reached an appropriate doughy consistency, it was carefully packed into the mold. A special nonmetallic flask was then subjected to manual pressure and placed inside the microwave oven for 3 min at 500 W for curing. Post curing, the flask was removed from the microwave and till achieving room temperature for 30 min. Subsequently, it was dipped in cold water for 20 min to facilitate deflasking. Deflasking was carried out by gently tapping the flask to release the specimens, and finally, all the specimens were finished and polished.

Acrylic resin specimens of both group IB and IIB containing  $TiO_2NP$  (modified group).  $TiO_2NP$ powder was added (3% by weight) to the polymer of heat cured and microwave cured resins, respectively, by employing the weighing balance which was bought from Adam Equipment 124 precision weighing balance, UK in the Central Service Unit at National Research Centre, National Research Centre (NRC), Cairo, Egypt. Then specimens' curing of each subgroup was performed as that of group IA and IIA, respectively.

## Biological, physical, and mechanical properties assessments

#### Biological assessment

The isolated colonies of organisms were cultured in 5 mL of broth medium at 37°C and left to grow overnight (16–18 hours). Then, the cultures were diluted, and the organism inoculum size was standardized by measuring the absorbance at 600 nm "absorbance of 0.5 at 600 nm follows the McFarland standard, which indicates the number of bacteria within a given range to standardize microbial testing". The provided resins were inserted in 1 mL of PBS pH7.4 and incubated at 37°C for 30 minutes to enhance the release of active substance, followed by vigorous vortex stock solution. The released solution was centrifuged at 8000xg for 10 minutes and the pellet

was resuspended in 200  $\mu$ L of PBS and used for antimicrobial assessment. In petri dishes, the *E. faecalis* pathogen was cultured in Luria-Bertani (LB) broth. A150  $\mu$ l of the organism was gently spread throughout the plate, then dry any extra liquid solution. Each agar plate was divided into 5 sections and labelled with the corresponding used resin. 100  $\mu$ l of each tested sample was added to disc in the tested dish, and 100  $\mu$ l of the PBS was added as negative control. The plates were incubated inverted overnight or until cells have grown out completely. After 7 days, the area of inhibited bacterial growth was measured and scaled with a ruler. as displayed in Figure 1.

#### Physical assessment

In this study, color stability was utilized to assess the physical characteristics of acrylic resin. Acrylic resin specimens (N=10 in each group) were immersed in tea. Color stability evaluation for each specimen was recorded pre and 7 days post of their immersion in prepared tested solution via utilizing spectrophotometer (Neu-Isenburg, Germany), as displayed in Fig. 2.

The color changes of each group were calculated via employing the C.I.E. L, a, b, uniform color scale. The magnitude of total color alteration was formulated by:  $\Delta E [\Delta E = (2\Delta L+2\Delta a+2\Delta b)^{1/2}] [27]$ , where  $\Delta E$  is degree of color change,  $\Delta L$  is L specimen – L standard,  $\Delta a$  is a specimen – a standard and  $\Delta b$  is b specimen – b standard.

#### Figure 1



Shows agar diffusion assay illustrated the antimicrobial potential of all groups on *Enterococcus faecalis*. (a) negative control group, (b) Group IA, (c) Group IB, (d) Group IIA, (e) Group IIB.

#### Figure 2



Shows spectrophotometer used in evaluation of color changes.

#### Mechanical properties assessment

The modulus of elasticity was measured using a Universal testing machine(INSTRON, 3345 England.). The specimens were centered on the machine's supports and gradually applied perpendicular to the midpoint of the strips. The load was recorded until the maximum capacity was reached. Load-deflection curves were recorded using computer software(Bluehill v1.5; Instron Ltd).. The elastic modulus was calculated using the formula E =FL3 /4Ybd3, where E represents the flexural modulus, F represents the maximum load, L represents span length, Y represents recorded deflection, b represents specimen width, and d represents specimen thickness, as displayed in Figure 3 [28].

#### Statistical analysis

All data were collected, revised, coded, and entered to the Statistical Package for Social Science (IBM SPSS) version 23. The quantitative data were presented as mean and standard deviations. To evaluate the data's normality, both the Shapiro-Wilk test and Kolmogorov test were employed, and it was observed that all data from the various groups exhibited a normal distribution. Subsequently, the comparison between two groups was done using Student *t*-test, while the comparison among the different groups was driven employing the one-way ANOVA test, accompanied by the Tukey Post Hoc test to facilitate several comparisons. A significance threshold of P less than 0.05 was adopted.

Figure 3



Shows evaluation of acrylic resin specimen modulus of elasticity through universal testing machine.

#### Results

#### **Biological assessment**

Regarding the bacterial evaluation of denture base materials specimens using the inhabitation zone of E. faecalis, the comparison between all groups displayed highly significant difference at P value less than 0.01. In addition, the multiple comparisons showed that negative control and control groups of conventional heat cured PMMA (group IA) and microwave cured PMMA (group IIA) were displayed no inhibition zone, while group of heat cured specimens modified with 3% TiO<sub>2</sub>NP (group IB) exhibited  $2.5 \pm 0.12$  mm, which was significantly lower (P < 0.05) than Microwave cured specimens modified with 3% TiO<sub>2</sub>NP (group IIB), which revealed  $2.5 \pm 0.22$  mm, as presented in Table 1.

#### **Physical assessment**

Regarding the color changes  $\Delta E$  of denture base materials specimens using spectrophotometer, comparison between different groups revealed highly significant difference as < 0.0001. In addition, the multiple comparisons revealed that the control groups of conventional heat cured PMMA (group IA) which exhibited  $18.07 \pm 6.27$  and microwave PMMA (group IIA) which exhibited cured  $28.2 \pm 2.99$  demonstrated the highest color changes. Followed by heat cured specimens modified with 3%  $TiO_2NP$  (group IB) which exhibited 13.18 ± 1.29. On the other hand, Microwave cured specimens modified with 3% TiO<sub>2</sub>NP (group IIB)  $6.78 \pm 0.51$  revealed the least amount of color changes, as presented in Table 2.

Table 1	Inhibition zone	(mm)	) regarding	Enterococcus	faecalis in	all grou	ps and sub	groups
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Inhibition Zone (mm)	М	SD	P-value
Negative control	0.0 <sup>a</sup>	0.0	
Group IA			
Conventional heat cured PMMA	0.0 <sup>a</sup>	0.0	
Group IB			
Heat cured PMMA incorporated with 3 % by weight TiO <sub>2</sub> NP	2.5 <sup>b</sup>	0.12	< 0.0001*
Group IIA			
Microwave cured PMMA	0.0 <sup>a</sup>	0.0	
Group IIB			
Microwave cured PMMA incorporated with 3 % by weight $TiO_2NP$	4.5 <sup>c</sup>	0.22	

All data with different superscript letters (a, b, c) were significantly different at *P-value* less than 0.05, using ANOVA. M: mean, PMMA: polymethyl meth acrylate, SD: standard deviation,  $TiO_2NP$ : titanium oxide nanoparticles. Significant difference as *P* less than 0.05.

Table 2	Color changes	in control and	l modified q	roups with 3	3% TiO <sub>2</sub> NP	using spectrop	photometer
						. J.	

Color changes ( $\Delta$ E)	Mean	SD	Р
Group IA			
Conventional heat cured PMMA	18.07 <sup>a</sup>	6.27	
Group IB			
Heat cured PMMA incorporated with 3% by weight TiO <sub>2</sub> NP	13.18 <sup>b</sup>	1.29	< 0.0001*
Group IIA			
Microwave cured PMMA	282 <sup>c</sup>	2.99	
Group IIB			
Microwave cured PMMA incorporated with 3% by weight $\text{TiO}_2\text{NP}$	6.78 <sup>d</sup>	0.51	

All data with different superscript letters (a, b, c) were significantly different at P value less than 0.05, using ANOVA. M: mean, PMMA: polymethyl meth acrylate, SD: standard deviation, TiO<sub>2</sub>NP: titanium oxide nanoparticles. \*Significant difference as P less than 0.05.

#### Mechanical properties assessment

Regarding modulus of elasticity of denture base materials specimens using universal testing machine, comparison between different groups revealed highly significant difference as P=0.0001. In addition, the multiple comparisons revealed that the controls groups of conventional heat cured PMMA (group IA) which exhibited 217.59 ± 32.88 and microwave cured PMMA (group IIA) which exhibited 218.14 ± 50.31 demonstrated the the lowest modulus of elasticity. Followed by heat cured specimens modified with 3% TiO<sub>2</sub>NP (group IB) which exhibited 311.8 ± 51.18. On the other hand, microwave cured specimens modified with 3% TiO<sub>2</sub>NP (group IIB) 740.5 ± 43.26 revealed the highest modulus of elasticity, as presented in Table 3.

#### Discussion

In this study, we meticulously considered various parameters in the experimental setup. We prepared metal specimens with precise shapes and dimensions to serve as molds for creating acrylic resin specimens, aligning with ADA specification no.12 [29]. For heat cured acrylic resin specimens, the conventional method was followed using a metal flask, while for microwave cured ones, a special microwave plastic flask was employed to avoid potential issues from microwave energy reflection [16,30].

All acrylic resin denture base materials in each group were manufactured following the recommended

	Table 3	Modulus of elast	city in contro	I and modified	groups with 3%	TiO <sub>2</sub> NP usin	g Universal testing	g machine
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Storage modulus	Mean	SD	Р
Group IA			
Conventional heat cured PMMA	217.59 <sup>a</sup>	32.88	
Group IB			
Heat cured PMMA incorporated with 3% by weight TiO <sub>2</sub> NP	311.8 <sup>b</sup>	52.18	0.0001
Group IIA			
Microwave cured PMMA	218.14 <sup>a</sup>	50.31	
Group IIB			
Microwave cured PMMA incorporated with 3% by weight TiO <sub>2</sub> NP	740.5 <sup>c</sup>	43.26	

All data with different superscript letters (a, b, c) were significantly different at *P* value less than 0.05, using ANOVA. M: mean, PMMA: polymethyl meth acrylate, SD: standard deviation,  $TiO_2NP$ : titanium oxide nanoparticles. \*Significant difference as *P* less than 0.05.

guidelines to ensure top-quality results. To enhance the PMMA's biocompatibility, stability, antifungal properties, and mechanical strength  $TiO_2NP$  was introduced, specifically using the anatase form for its superior photocatalytic activity and stability [31,32]. To minimize any potential negative effects of  $TiO_2NP$ , especially with increased concentrations, we added only 3% by weight to the two different PMMA resin types used for specimen fabrication [33].

E. faecalis was chosen for the biological assessment of PMMA resins in this study due to its versatile nature. It is considered one of the most strong and prevalent intraoral species, capable of existing as both single cocci and in chains [20,34]. In addition, it exhibits broadspectrum colonization within the oral cavity and is associated with oral mucosal lesions, particularly in immunocompromised patients. In terms of the physical properties of acrylic resin, we focused on color stability, as it plays a significant role in esthetics and patient satisfaction [35,36]. To ensure precision, we utilized a spectrophotometer, and consistency was evaluated employing the Commission International de L'Eclairage uniform color scale (CIE scale) [37]. Furthermore, this research assessed the viscoelastic properties using a constant frequency of 1 Hz and a temperature range of 5-50°C to simulate masticatory rhythms and the intraoral temperature conditions, ensuring that the resins perform well in clinical settings [38].

Outcomes of this research displayed that both group IA and G IIA (without  $TiO_2NP$ ) showed no inhibition zone, while in modified groups (with  $TiO_2NP$ ) revealed inhibition zone. This might be related to the  $TiO_2NP$  which is an effective antimicrobial additive due to its chemical stability, non-toxicity, and ability to inhibit pathogenic bacteria adherence. PMMA resin reinforcement with 3%  $TiO_2NP$  significantly reduces microbial count and attachment, particularly *E. faecalis*, and enhances their biological properties.

On the other hand, group IIB showed higher inhibitory zone than group IIA, this might be attributed to the different nature of both acrylic resin and their variance in the curing method as proved by other studies [9,10]. Moreover, microwave curing offers distinct advantages over water bath curing due to its rapid temperature increase and heating capabilities. When using water baths, the formation of bubbles occurs, which can become trapped within the polymer matrix, leading to excessive porosity and surface irregularities [39,40]. Conversely, heat cured PMMA specimens tend to harbor more bacteria and facilitate their colonization, thereby reducing their ability to create a sufficient inhibitory zone [41].

The current research findings revealed that both the control and modified heat cured PMMA groups had the uppermost span of color variations group IA ( $18.07 \pm 6.27$ ) and group IIA ( $28.2 \pm 2.99$ ), succeeded by the control microwave cured acrylic resin group IB ( $13.18 \pm 1.29$ ), then the modified microwave one group IIB ( $6.78 \pm 0.51$ ) displayed the least amount of color changes individually.

This can be attributed to the fact that color stability is influenced not only by the type of PMMA resin but also by the processing method, chemical composition, and specific standards applied. Certain studies have explored how the processing method can impact the degree of color stability during the PMMA's polymerization process [9,10]. It has been noted that shorter processing times result in better color stability. As a result, since microwave polymerization only takes around 3 min. compared to the 9 hours required for the hot water bath technique, the color stability of the PMMA specimens was significantly better in the microwave cured ones compared to those cured through heat [42,43]. The study found that adding 3% TiO<sub>2</sub>NP to PMMA resin types reinforces the microwave polymer matrix, limiting color changes and absorbing more amount of light than the control specimens, resulting in more opaque and glossier specimens [8,44].

Findings of this research, concerning the modulus of elasticity in mechanical properties evaluation, revealed insignificant difference between both control groups of heat cure PMMA group IA which exhibited the lowest modulus of elasticity and microwave one group IIA. followed by modified heat cured PMMA group IB, while modified microwave cured PMMA group IIB displayed the highest storage modulus. The flexural strength and modulus of acrylic resin were greatly enhanced by the incorporation of TiO<sub>2</sub> NPs. TiO<sub>2</sub> NP addition results in a concentration-dependent process which increases flexural strength and modulus. Physically rigid particles, TiO2 NPs can fill voids in the material's matrix. They may absorb energy and stresses before failure if they are distributed properly. Because they are physically inflexible, TiO<sub>2</sub> nanoparticles can be very effective at absorbing energy from the material. These nanoparticles can assist in more uniformly distributing stresses throughout the matrix when external pressures or stresses are applied to the material. They serve as stress absorbers, lowering the concentration of tension at particular locations. Additionally, the greatest flexural strength and modulus were produced by lower concentrations of  $TiO_2$  NPs, which is consistent [45,46].

#### Conclusion

Within the limitation of the present study, it can be concluded that both heat and microwave cured acrylic resins have a comparable mechanical feature regarding the modulus of elasticity; as well as absence of antibacterial properties. Microwave cured resin has a lower color stability property compared to that of heat cured resin. Adding 3% TiO2NP to heat and microwave cured resins induced antibacterial characteristics against *E. faecalis.* Besides improvement of the evaluated mechanical (modulus of elasticity) and color stability properties.

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#### **Conflicts of interest**

There are no conflicts of interest.

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# Antihyperlipidemic and cardiopreventive properties of Arabic gum in nicotinamide/streptozotocin-induced diabetic rats

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#### Background/Aim

Diabetes mellitus (DM) and its complications have a negative impact on human health and the economy worldwide. Diabetic patients are at a high risk of dying from cardiovascular diseases. Arabic gum (AG) is a natural product that exhibits potent anti-inflammatory, antioxidant and hypoglycemic properties. The purpose of this study was to scrutinize the antihyperlipidemic and cardiopreventive efficacy and to assess the antioxidant and anti-inflammatory roles of AG in nicotinamide (NA)/ streptozotocin (STZ)-induced DM in rats.

#### Material and methods

Three groups of 18 adult (6 each) male Wistar rats each were used for the experiment. The first group was the normal control group, which received 0.9% NaCl daily by oral gavage for 8 weeks. The rats in the second group were injected with 60 mg/kg b.w. STZ in citrate buffer (pH 4.5) intraperitoneally (IP), after being given intraperitoneally 120 mg/kg b.w. NA. They also received 0.9% NaCl daily by oral gavage for 8 weeks. The third group was treated with 20 mg AG/kg b.w./day suspended in 0.9% NaCl by oral gavage for 8 weeks after inducing DM in the same way as the second group.

#### Results

Hyperglycemia and hyperlipidemia were observed in DM rats. They also had significantly higher levels (P<0.05) of serum creatine kinase (CK), creatine kinase myocardial band (CK-MB), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH), which indicate heart dysfunction. The diabetic heart suffered from oxidative stress, as shown by significant increases (P<0.05) of malondialdehyde (MDA) and decreased glutathione (GSH), glutathione peroxidase (GPx), and superoxide dismutase values (SOD). AG treatment improved blood glucose and serum lipid levels, as well as heart function biomarkers in serum. AG also reduced oxidative stress and enhanced antioxidant defenses in the diabetic heart. Immune-inflammatory markers, such as nuclear factor-kappa B and tumor necrosis factor- $\alpha$  and apoptotic protein p53 expressions were elevated in diabetic rats (P<0.05) markedly, but the treatment with AG exhibited normal levels for them.

#### Conclusion

In conclusion, this study demonstrated that AG has a preventive role against heart injury in NA/STZ-induced DM in rats. AG improved the metabolic, oxidative, and inflammatory status as well as apoptosis and their cardiac function in diabetic rats. Moreover, AG improved the histological picture of cardiac myocytes and therefore, it may be a potential natural remedy for diabetic cardiomyopathy.

#### Keywords:

antioxidant, Arabic gum, diabetes mellitus, hyperglycemia, hyperlipidemia

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#### Introduction

Cardiovascular problems are a major cause of death and disability among diabetic patients worldwide due to the long-term effects of hyperglycemia [1]. Diabetes mellitus (DM) is caused by a metabolic imbalance of proteins, lipids, and carbohydrates at the homeostasis level. DM is classified into two types: type I diabetes (T1DM), characterized by insufficient insulin production by the pancreas and type II diabetes (T2DM), characterized by insulin resistance of the body tissues [2]. The World Health Organization (WHO) reports that chronic DM is rising rapidly worldwide, with a doubling of cases from 1980 to 2014. Also, about half of the deaths were due to high blood sugar and occurred before the seventh decade of life [3]. Oxidative stress has a key role in DM complications, as it increases the ratio of oxidants to antioxidants [4], which damages biological macromolecules (proteins, carbohydrates, fats, and nucleic acids). This leads to more reactive oxygen species and more cell damage. DM induces

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alterations in the cardiac muscle's structure and function, leading to a condition called diabetic cardiomyopathy (DCM). DCM has a complex origin, with many interrelated mechanisms responsible for cardiomyopathy in DM [5]. Oxidative stress and inflammation are thought to play a key role in the worsening of DCM. Oxidative stress is a major factor that leads to the development and deterioration of DCM in the DM heart [6]. DM complications are generally seen as the outcome of oxidative stress. Moreover, DM complications are linked to the inflammatory response and have been found to be faster at high blood sugar levels, which trigger adipocyte acute response factor creation [7].

Arabic acacia gum (AG) is a food additive that has many uses in food production and is also called *Acacia senegal* L [8]. It can act as a carrier for many drugs that treat patients and can also reduce the toxicity of some drugs. Besides, it has antioxidant and antiinflammatory effects that make AG the subject of interest for studying human diseases that involve inflammation and inflammatory pathways [9–12]. AG is used for its potential therapeutic effects in many diseases, such as metabolic disorders, gastrointestinal disorders, periodontitis, rheumatoid arthritis and sickle cell disease [13].

AG is widely used and considered safe in both folk medicine and pharmaceutical products. It is indigestible by humans or animals, but it can undergo fermentation in the colon to generate shortchain fatty acids, which may have various health benefits. It has a prebiotic effect as it increases the levels of beneficial bacteria such as Bifidobacteria, Lactobacteria, and Bacteroides. Furthermore, it also has anticancer, antioxidant effects and can protect the liver and the heart from damage [14].

Consequently, the purpose of this study was to explore how AG can modulate heart function, antioxidant defense mechanisms and the immunoinflammatory pathway, thus treating DCM in rat models.

#### Materials and methods Plant materials

AG was purchased (in powder form) from Qualikems Fine Chem PVT. LTD (India).

#### Chemicals

Nicotinamide (NA) and streptozotocin (STZ) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

#### **Experimental animals**

The present study involved adult male Wistar rats (120-140 g) that were obtained from the Egyptian Organization for Biological Products and Vaccines (VACSERA), Helwan Station, Cairo, Egypt. The animals were observed for 2 weeks before the experiment to ensure they were free of infections. They were housed in the animal facility of the Zoology Department, Faculty of Science, Beni-Suef University, Egypt, in well-ventilated polypropylene cages. They were exposed to a normal light-dark (10-12 h/day),cvcle a normal temperature (20-25°C), and had access to food and water at all times.

#### Ethical consideration

All the experiments were done in compliance with the public health guide for the care and use of laboratory animals and followed the ethical consideration of the Experimental Animal Ethics Committee of the Faculty of Science, Beni-Suef University, Egypt under approval number BSU/FS/2018/26. The number of animals was minimized and all efforts were made to reduce their pain, distress and discomfort.

#### Induction of diabetes mellitus

T2DM was induced in 16-h-fasted rats according to Aziz et al. [15] and Ahmed et al. [16] by a single intraperitoneal (i.p.) injection of STZ (60 mg/kg b.w.) dissolved in 0.09 M citrate buffer (pH 4.5), 15 min after the i.p. injection of nicotinamide (120 mg/kg b.w.) prepared in 0.9% saline solution. Rats received 5% glucose in drinking water to overcome the hypoglycemia caused by STZ. Ten days after the STZ injection, overnight fasted animals were given glucose (3 g/kg b.w.) by gastric intubation. After 2 h of oral glucose loading, blood samples were withdrawn from the lateral tail vein, left to coagulate and centrifuged, then the serum glucose concentration was measured. Rats that had a serum glucose of 180-300 mg/dl after 2 h of glucose intake were considered mild DM and were included in the experiment.

#### Phenolic constituents of Arabic gum by highperformance liquid chromatography (HPLC) Sample preparation

A sample was prepared by dissolving 20 mg of Arabic gum in 1 ml of 80% methanol (vortex, sonication, filtration and injection).

#### HPLC condition

HPLC analysis was carried out in Chromatography Lab., Central Labs., National Research Centre using

an Agilent 1260 series. The separation was carried out using a Zorbax Eclipse plus C8 column (4.6 mm×250 mm i.d.,  $5\mu$ m). The mobile phase consisted of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) at a flow rate of 0.9 ml/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (82% A), 0–1 min (82% A), 1–11 min (75% A), 11–18 min (60% A), 18–22 min (82% A) and 22–24 min (82% A). The multiwavelength detector was monitored at 280 nm. The injection volume was 5 µl for each of the sample solution. The column temperature was maintained at 40°C.

#### Experimental design

Experimental animals were allocated into three groups, each comprising six rats, as follows:

- (1) Group I (normal control): rats were given the equivalent volume of isotonic solution (0.9% NaCl) daily by oral gavage for 8 weeks.
- (2) Group II (NA/STZ-induced diabetic control): rats were orally given the equivalent volume of isotonic solution daily through oral gavage for 8 weeks.
- (3) Group III (diabetic + AG treated group 'NA/ STZ-induced diabetic group treated with Arabic gum') diabetic animals received 20 mg/kg b.w./day AG suspended in 0.9% NaCl by oral gavage for 8 weeks [17].

#### Sampling

At the end of the treatment period, the rats were deprived of food overnight and were anesthetized by diethyl ether inhalation. Blood samples were collected from the jugular vein and then the rats were killed by sudden cervical decapitation while they were anesthetized. Blood was left at room temperature to coagulate and then centrifuged at 3000 rpm for 15 min. The clear, non-hemolyzed supernatant sera were collected into three Eppendorf tubes for each rat and kept at  $-80^{\circ}$ C until used.

#### Preparation of tissue homogenates

Tissue homogenates were prepared as follows: rats were killed; heart tissue  $(5\mu m)$  was cut and rinsed with ice-cold (0.01 M, pH=7.4) phosphate-buffered saline (PBS), weighed, minced and then homogenized in PBS (9 ml PBS per gram tissue) with a glass homogenizer on ice. The homogenates were centrifuged at 3000 rpm. for 10 min and the supernatants were removed and frozen at  $-80^{\circ}\text{C}$ .

#### Biochemical analyses

Spectrophotometrically, using kits purchased from SPINREACT serum glucose was estimated

according to Valerie [18]. Serum insulin level was measured using sandwich enzyme-linked immunosorbent assay (ELISA) using kits purchased from Linco Research, USA, in accordance with the manufacturer's instructions. Creatine kinase (CK) and creatine kinase myocardial band (CK-MB) were estimated according to Clayton and Thomas [19] and Wu and Bowers [20]. Lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) were estimated following the methods of Young [21] and Reitman and Frankel [22], respectively, using kits purchased from SPINREACT Co. (Barcelona, Spain). Serum total cholesterol, triglycerides (TG), low-density lipoprotein cholesterol (LDL-cholesterol) and highdensity lipoprotein cholesterol (HDL-cholesterol) were estimated using kits of SPINREACT Co. (Barcelona, Spain) following the methods of Burtis et al. [23], Buccolo et al. [24], Armstrong and Seidel [25] and Jacobs et al. [26], respectively.

Measurement of oxidative stress and antioxidant biomarkers Spectrophotometrically, the heart level of reduced glutathione (GSH) was measured according to Beutler peroxidation/ et al. [27]; lipid malondialdehyde (LPO/MDA) level was measured according to Ohkawa et al. [28]; glutathione peroxidase (GPx) activity was determined following the method of Paglia and Valentine [29] and superoxide dismutase (SOD) activity was estimated according to Marklund and Marklund [30] using kits purchased from Biodiagnostic Co. (Egypt).

## Estimation of mRNA expression of heart NF- $\kappa$ B, TNF- $\alpha$ , and p53

Total RNA from the liver tissue was extracted according to the method of Chomzynski and Sacchi [31] using the Thermo Scientific GeneJET RNA purification kit obtained from Thermo Fisher Scientific Inc., Rochester, New York, USA. The purified RNA was transcribed into cDNA and then amplified in a PCR reaction using the Thermo Scientific Verso 1-Step real-time polymerase chain reaction Reddy Mix Kit (Thermo Fisher Scientific Inc., Rochester, New York, USA) in the presence of specific primers (Table 1) obtained from Biosearch Technologies, South McDowell Blud and Petaluma, CA, USA [32-35]. Real-time polymerase chain reaction products were electrophoresed on a 1.5% agarose gel and visualized under ultraviolet light ethidium after staining with bromide. The electrophoretic picture was analyzed by the gel documentation system (GelDocu Advanced) and the values of nuclear factor-kappa B (NF-kB) according to Shaker and Sourour [32]. Tumor necrosis factor- $\alpha$
mRNA species	Forward primer sequence	Reverse primer sequence	Reference numbers
NF-κB	5'd TACCATGCTGTTTTGGTTAC3'	5'd dTCAAGCTACCAATGACTTTC 3'	[32]
TNF-α	5'd GCTGAGGTTGGACGGATAAA3'	5'd AAAATCCTGCCCTGTCACAC3'	[33]
P53	5'd-GCTGCCCTCCCTTC TCCTAG3'	5′d CCCCGACTTTGGAGTAGTCTGA3′	[34-36]
β-actin	5'd TCACCCTGAAGTACCCCATGGAG3'	5'd TTGGCCTTGGGGTTCAGGGGG 3'	[32]

#### Table 1 Primer sequences for polymerase chain reaction

(TNF- $\alpha$ ) and apoptotic protein p53 expression were normalized to the quantity of  $\beta$ -actin [32–36].

## Histological examination

After embedding the heart tissues in paraffin blocks, serial sections (4  $\mu$ m thick) were mounted on glass slides, washed in a water bath and left in an oven for dewaxing. Finally, the sections were stained with hematoxylin and eosin [37]. Histological changes were assessed under an electrical light microscope (Olympus CX 41 RF, TOKYO, JAPAN). Adobe Photoshop version 8.0 was used for processing the photomicrographs.

## Statistical analysis

All data were coded, tabulated and statistically analyzed using the Statistical Package for the Social Sciences software, SPSS version 24. All data are presented as mean±standard error. The obtained data were analyzed by post hoc one-way analysis of variance followed by Tukey's methods [38]. Data were considered statistically significant when the *P*-value is less than or equal to 0.05.

#### Figure 1

# Results

## Phenolic profile of Arabic gum by HPLC

HPLC analysis of Arabic gum, illustrated in Table 2 and Fig. 1, revealed 10 phenolic compounds, consisting of six phenolic acids and/or compounds (gallic acid, chlorogenic acid, caffeic acid, syringic acid and vanillin) and four flavonoids (catechin, rutin, naringenin and quercetin). Chlorogenic acid was the most abundant in Arabic gum, followed by catechin, rutin, gallic acid and

Table 2 Phenolic compounds identified in Arabic gum using
high performance liquid chromatography

Polyphenols	Area	Conc. (µg/ml)	Conc. (µg/g)
Gallic acid	23.70	2.11	105.50
Chlorogenic acid	59.10	8.00	400.18
Catechin	17.36	3.95	197.41
Caffeic acid	1.18	0.09	4.72
Syringic acid	25.32	1.93	96.59
Rutin	18.42	3.09	154.36
Vanillin	7.88	0.30	14.86
Ferulic acid	1.53	0.09	4.61
Naringenin	3.28	0.31	15.53
Quercetin	1.24	0.17	8.25



syringic acid. However, vanillin, naringenin, quercetin, ferulic acid and caffeic acid were found in minor concentrations.

## Arabic gum improves lipid profile

As far as we know, the current study investigates the effect of Arabic gum (AG) on heart damage in T2DM. DM caused significant increases in serum levels of total cholesterol, triglycerides, LDL cholesterol and the ratios of total cholesterol to HDL-cholesterol and LDL cholesterol (P<0.05), while HDL-cholesterol levels decreased significantly compared with the control group. However, these changes were reversed when the DM rats received AG treatment, as the serum levels of the above parameters decreased significantly (P<0.05) except for HDL-cholesterol, which increased significantly compared with the DM group (Table 3).

## Arabic gum enhances glucose control and heart efficacy

Back to the present results, fasting blood glucose levels and cardiac damage markers (serum CK, CK-MB, LDH and AST) were higher ( $P \le 0.05$ ) in DM rats than in the control group. AG treatment significantly  $(P \le 0.05)$  reduced fasting glucose and cardiac damage markers levels of CK-MB, LDH and AST in the blood relative to the DM group (Table 4).

# Arabic gum attenuates oxidative stress and enhances the antioxidant defense system

DM significantly ( $P \le 0.05$ ) decreased heart values of GSH, GPx and SOD, which are antioxidants, relative to the control group. However, AG treatment significantly increased the heart values of these antioxidants, compared with the diabetic group. Diabetic rats also increased markedly ( $P \le 0.05$ ) the heart level of MDA, which is a marker of oxidative stress, in contrast to the control group. However, AG treatment significantly reduced the heart level of MDA, relative to the diabetic group (Table 5).

## Arabic gum reduces heart inflammation

Diabetes elevated heart mRNA expressions of NF- $\kappa$ B and TNF- $\alpha$ , which are inflammatory factors, above normal ( $P \le 0.05$ ), in contrast to the control group. However, AG treatment lowered the mRNA expression of NF- $\kappa$ B and TNF- $\alpha$  below normal ( $P \le 0.05$ ), relative to the DM group. Meanwhile,

Table 3 Influence of Arabic gum on serum lipid profile in normal and diabetic rats

Group parameters	G1 (negative control)	G2 (diabetic control)	G3 (diabetic+AG treated)		
Total cholesterol (mg/dl)	49.1±0.9 <sup>a</sup>	101.2±2.0 <sup>b</sup>	57.02±0.6 <sup>a</sup>		
Triglycerides (mg/dl)	58.3±2.9 <sup>a</sup>	97.8±2.9 <sup>b</sup>	62.08±3.1 <sup>a</sup>		
HDL-cholesterol (mg/dl)	28.5±1.1 <sup>a</sup>	11.4±0.4 <sup>b</sup>	28.1±1.8 <sup>a</sup>		
LDL-cholesterol (mg/dl)	7.0±1.7 <sup>a</sup>	39.0±1.1 <sup>b</sup>	18.9±1.1 <sup>c</sup>		
Total chol/HDL-cholesterol	1.7±0.5 <sup>a</sup>	8.9±0.4 <sup>b</sup>	2.03±0.1 <sup>a</sup>		
Total chol/LDL-cholesterol	7.01±0.4 <sup>a</sup>	2.3±0.4 <sup>b</sup>	3.02±0.08 <sup>a</sup>		

All data are presented as mean $\pm$ SE. All means with different superscript letters (a, b, c) within the same row are significantly different at *P* less than 0.05.

<b>Fable 4 Influence of Arabic gum</b>	on serum glucose leve	and cardiac function	markers in normal and	d diabetic rats
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Group parameters	G1 (negative control)	G2 (diabetic control)	G3 (diabetic+AG treated)
Fasting glucose (mg/dl)	81.3±3.2 <sup>ª</sup>	472.5±3.9 <sup>b</sup>	102.2±0.6 <sup>c</sup>
Creatine kinase (IU/I)	47.0±4.3 <sup>a</sup>	544.2±6.2 <sup>b</sup>	69.5±4.5 <sup>°</sup>
Creatine kinase-MB (IU/I)	82.3±0.3 <sup>ª</sup>	276.5±0.9 <sup>b</sup>	95.5±0.6 <sup>c</sup>
LDH (IU/I)	477.6±4.2 <sup>a</sup>	$1558.5 \pm 8.4^{b}$	493.3±5.3 <sup>a</sup>
AST (IU/I)	22.7±4.8 <sup>a</sup>	149.0±9.0 <sup>b</sup>	42.1±4.8 <sup>c</sup>

All data are presented as mean $\pm$ SE. All means with different superscript letters (a, b, c) within the same row are significantly different at *P* less than 0.05.

Table 5	Influence of	f Arabic gum	on the hear	t oxidative stress	(MDA) and	antioxidant	defense (G	GSH, GPx and	SOD) in	normal an	d
diabetic	rats										

Group parameters	G1 (negative control)	G2 (diabetic control)	G3 (diabetic+AG treated)	
GSH (mol/100 mg tissue)	121.7±0.57 <sup>a</sup>	88.9±4.7 <sup>b</sup>	116.9±5.1 <sup>a</sup>	
GPx (mU/100 mg tissue)	86.2±0.10 <sup>a</sup>	71.8±2.1 <sup>b</sup>	85.0±0.25 <sup>a</sup>	
SOD (U/g)	63.6±1.8 <sup>a</sup>	33.7±0.79 <sup>b</sup>	59.2±2.1 <sup>a</sup>	
MDA (nmol/ 100 mg tissue/h)	25.8±2.6 <sup>a</sup>	139.3±2.7 <sup>b</sup>	25.1±1.3 <sup>a</sup>	

All data are presented as mean±SE. All means with different superscript letters (a, b, c) within the same row are significantly different at P less than 0.05.

Groups Parameters	G1 (negative control)	G2 (diabetic control)	G3 (diabetic +AG treated)
NF-κB (fold change)	1.05±0.01 <sup>a</sup>	4.26±0.03 <sup>b</sup>	1.9±0.04 <sup>°</sup>
TNF- $\alpha$ (fold change)	1.01±0.01 <sup>a</sup>	6.22±0.005 <sup>b</sup>	1.12±0.004 <sup>c</sup>
P53 (fold change)	1.11±0.01 <sup>a</sup>	7.11±0.003 <sup>b</sup>	1.7±0.003 <sup>c</sup>

Table 6 Influence of Arabic gum on the heart mRNA expression of inflammatory markers (NF- $\kappa$ B and TNF- $\alpha$ ) and apoptotic protein (P53) in diabetic rats

All data are presented as mean $\pm$ SE. All means with different superscript letters (a, b, c) within the same row are significantly different at *P* less than 0.05.

there was no significant difference in heart mRNA expression of P53, which is a tumor suppressor and apoptotic protein, among the control, DM and AG-treated groups (Table 6).

## **Histopathological findings**

The heart sections of normal control depicted normal histological structure of the heart as the cardiac muscle fibers are aligned with each other with an oval central nucleus (Fig. 2a). The heart section of diabetic rats, however, depicted focal necrosis of cardiac myocytes associated with inflammatory cell infiltration (Fig. 2b)

### Figure 2

and vacuolation of the wall of blood vessel associated with perivascular edema and inflammatory cell infiltration (Fig. 2c). The diabetic rats treated with AG extract exhibited mild congestion of myocardial blood vessels in the heart of some animals and nearnormal histological structures in others (Fig. 2d and e).

## Discussion

One of the main causes of death among DM patients in developing countries is damage to the cardiovascular system caused by long-term high blood sugar levels,



Heart of a rat from a normal control (A) showing the normal histological structure of cardiac myocytes. In the heart of a diabetic rat (B), focal necrosis of cardiac myocytes was associated with inflammatory cells infiltration (black arrows). In addition to vacuolation (C) of the wall of the blood vessel associated with perivascular edema and inflammatory cell infiltration (black arrows). The heart section of a rat from the diabetic group treated with Arabic gum showed congestion (D) of myocardial blood vessels (black arrows). However, no pathological alterations were observed (E) in the heart tissue of a diabetic rat treated with Arabic gum (H and E ×400).

which leads to a condition called cardiomyopathy [39]. According to Jia et al. [40], high blood sugar levels are linked to an enlargement of the heart due to problems in the small blood vessels. This happens because longterm DM makes the heart less responsive to insulin [41], increases oxidative stress and impairs the calcium transport in the mitochondria, which produces energy in the cells [42]. A recent study suggested that GA could slow down the progression of DCM by improving high blood sugar levels, high blood lipid levels and oxidative stress. This implies that GA might have the potential to treat DCM in humans [43]. DCM is a condition where the heart muscle and function are abnormal in people with DM, even when they do not have other factors that can affect the heart, such as blocked arteries, high blood pressure, or significant valve problems [39]. Early management and treatment of high blood sugar can improve the quality of life of diabetic patients and prevent complications. GA lowers blood sugar by increasing the amount of stool, decreasing its water content, trapping bile acids and improving the body's functions [44].

DM raised serum levels of total cholesterol, triglycerides, LDL cholesterol, and their ratios and lowered HDL-cholesterol levels, compared with the control group. AG treatment reversed these changes, except HDL-cholesterol increased, in contrast to the DM group. The present study was in line with Fouda et al. [43], who reported that broiler chickens had lower serum cholesterol and triglycerides when fed diets with 5% and 7.5% AG, which has a hypolipidemic effect. Mice that had a fat-rich diet and 7% GA for about four-and-a-half months also had lower serum cholesterol and triglycerides. Different mechanisms have been proposed for the hypolipidemic action of AG. One way is that GA increases bile salt excretion in the stool, which makes the liver use more cholesterol to make new bile salts and reduces body fat and serum cholesterol [45,46]. Another way is that GA changes the expression of genes that are related to cholesterol formation and fat oxidation in mice [47]. Jangra et al. [48] also suggested that GA increases the expression of the fasting-induced adipose factor gene in the mice, which helps break down fat and prevent fat accumulation. The role of the fasting-induced adipose factor gene in regulating lipid metabolism in T2DM has been confirmed by other studies [49,50]. Some studies indicated that AG increases the viscosity of the intestinal content and therefore, prevents intestinal lipid absorption [51]. Another mechanism indicated that AG acts by interrupting the enterohepatic circulation of bile acids, resulting in increased bile acid excretion and subsequently

decreasing plasma cholesterol concentrations, in addition to enhancing the numbers of lipoprotein receptors in the liver and lowering plasma cholesterol concentrations [52]. Arabic gum lowered blood sugar levels significantly by stimulating the production of insulin from  $\beta$  cells. It did this by getting rid of free radicals and preventing the oxidation of lipids [53,54].

Returning to the current results, diabetic rats had higher fasting glucose levels and cardiac damage markers (CK, CK-MB, LDH, and AST) reflecting cardiomyopathy. AG treatment significantly lowered fasting blood glucose and cardiac damage markers, which were increased by DM. Heart section of diabetic rats, depicted focal necrosis of cardiac myocytes associated inflammatory with cell infiltration and vacuolation of the wall of blood vessels associated with perivascular edema and inflammatory cell infiltration. The diabetic rats treated with AG extract exhibited mild congestion of myocardial blood vessels in the heart of some animals and near-normal histological structures in others. The NA/STZ-induced histological changes were consistent with the biochemical evidence of enhanced oxidative stress manifested by lipid peroxidation and inflammation. These results are consistent with Upaganlawar and Balaraman [55], who reported that creatine kinase-MB, is a wellknown indicator of cardiomyocyte damage and that there is a positive association between the degradation of muscle filaments and increased serum levels of CK-MB. Al-Rasheed et al. [56] proved that serum CK-MB and AST were elevated in rats with experimentally induced Interestingly, DCM. AG treatment significantly lowered the levels of CK-MB, LDH and AST in the blood and prevented hyperglycemiainduced cardiomyocyte damage, demonstrating its cardioprotective benefit of AG. Fouda et al. [43] showed that rats with STZ-induced DM had significantly higher levels of hyperglycemia and hypoinsulinemia. This might be because STZ was selectively taken up by  $\beta$  cells through the glucose transporter GLUT2 and destroyed  $\beta$ -cells by damaging their nuclear DNA. STZ injection did not affect non-β endocrine cells in pancreatic islets, suggesting that STZ was specific to beta cells [57]. Pal et al. [54] demonstrated that Arabic gum had a significant hypoglycemic effect by stimulating the secretion of insulin from pancreatic beta cells by eliminating free radicals and suppressing lipid peroxidation.

DM lowered heart levels of antioxidants (GSH, GPx, and SOD) and raised oxidative stress markers (MDA)

significantly, compared with the control group. AG reversed these effects, boosted the immune system by increasing GSH level, GPx and SOD activities and inhibited oxidative stress by reducing MDA level, compared with the DM group. These results align with Fouda et al. [43], who proved that STZinduced DM, reduced SOD activities and increased MDA levels, indicating oxidative stress. This could be a reflection of impaired antioxidant defense potential, as hyperglycemia and hyperlipidaemia are linked to increased ROS production [58]. Arabic gum improved MDA and SOD values because it contains four antioxidant minerals: copper, iron, manganese and zinc, or because it positively influenced the expression of antioxidant enzymes [59]. The high blood lipid levels seen in STZ-induced DM rats might be caused by oxidative stress due to chronic hyperglycemia [60] or by insulin resistance, as insulin resistance is linked to hyperglycemia and changes in lipid metabolism [61].

DM increased heart mRNA expressions of inflammatory factors (NF- $\kappa$ B and TNF- $\alpha$ ) above normal, but AG treatment reduced them below normal, compared with the control group. Heart mRNA expression of P53, a tumor suppressor and apoptotic protein, was not significantly different among the groups. These results are in agreement with Nemmar et al. [62], who stated that Arabic gum reduced cardiotoxicity caused by water-pipe smoke exposure in mice by a mechanism that involved activating the nuclear factor-like 2 signaling pathway. Nuclear factor-like is an important transcription factor that has a key role in activating antioxidant enzymes to cope with oxidative stress [63]. Indira and Abhilash [64] illustrated that DM causes the activation of a protein called NF- $\kappa$ B in  $\beta$ -cells, which are the cells that produce insulin. NF- $\kappa$ B is triggered by different substances that cause inflammation and damage  $\beta$ -cells. When NF- $\kappa$ B is activated, it turns on a gene called inducible nitric oxide synthase, which makes a molecule called NO that kills  $\beta$ -cells. In T1DM, a substance called IL-1 $\beta$  activates NF- $\kappa$ B and causes the death of  $\beta$ -cells in the pancreas. In T2DM, NF- $\kappa$ B not only kills  $\beta$ -cells, but also makes them resistant to insulin. NF- $\kappa$ B also interacts with other substances, which increases its activity and causes more inflammation throughout the body. This inflammation leads to many complications of DM, such as the heart, eye, kidney and nerve problems. Therefore, blocking NF-KB could be a way to treat DM. When NF- $\kappa$ B is activated repeatedly, it blocks the action of inflammatory mediators, which helps the tumor grow. Many small molecules from natural or synthetic sources can affect different signalling pathways, including those involving NF- $\kappa$ B and p53, a protein that controls cell growth. These molecules have changed the way cancer is treated and managed. Some of the NF- $\kappa$ B inhibitors can fight cancer by making the cancer cells produce more p53 [65].

### Conclusion

These results suggest that Arabic gum has antihyperlipidemic and cardiopreventive properties in NA/STZ-induced DM rats and prevents cardiomyopathy by increasing antioxidants, decreasing oxidative stress, apoptosis and stopping the harmful effects of pro-inflammatory cytokines.

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#### **Conflicts of interest**

There are no conflicts of interest.

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