Spine 2.5 mm

ISSN : 2589-627X

Volume 1 • Issue 2 • July-December 2018

# Journal of Nature and Science of Medicine

www.jnsmonline.org

JNSM

The Official Publication of
The College of Medicine, King Saud University

Wolters Kluwer

Medknow

Journal of Nature and Science of Medicine • Volume 1 • Issue 2 • July-December 2018 • Pages 41-88

Print ISSN: 2589-627X, E-ISSN: 2589-6288

# Journal of Nature and Science of Medicine

**Official Publication of the College of Medicine, King Saud University**

## Editorial Board



## General Information

**The Journal**

Journal of Nature and Science of Medicine, a publication of College of Medicine, King Saud University, is a peer-reviewed print + online Semiannual journal. The journal's full text is available online at www.jnsmonline.org. The journal allows free access (Open Access) to its contents and permits authors to self-archive final accepted version of the articles on any OAI-compliant institutional / subject-based repository.

**Abstracting and Indexing Information**

The journal is registered with the following abstracting partners: Baidu Scholar, CNKI (China National Knowledge Infrastructure), EBSCO Publishing's Electronic Databases, Exlibris – Primo Central, Google Scholar, Hinari, Infotrieve, ProQuest, TdNet.

**Information for Authors**

There are no page charges for JNSM submissions. Please check http://www.jnsmonline.org/contributors.asp for details.

All manuscripts must be submitted online at www.journalonweb.com/jnsm

For mode of payment and other details, please visit www.medknow.com/subscribe.asp.

Claims for missing issues will be serviced at no charge if received within 60 days of the cover date for domestic subscribers, and 3 months for subscribers outside India. Duplicate copies cannot be sent to replace issues not delivered because of failure to notify publisher of change of address.

The journal is published and distributed by Wolters Kluwer India Private Limited. Copies are sent to subscribers directly from the publisher's address. It is illegal to acquire copies from any other source. If a copy is received for personal use as a member of the society/society, one cannot resale or give-away the copy for commercial or library use.

The copies of the journal to the members of the society are sent by ordinary post. The editorial board, society or publisher will not be responsible for non-receipt of copies. If any member/subscriber wishes to receive the copies by registered post or courier, kindly contact the publisher's office. If a copy returns due to incomplete, incorrect or changed address of a member/subscriber on two consecutive occasions, the names of such members will be deleted from the mailing list of the journal. Providing complete, correct and up-to-date address is the responsibility of the member/subscriber.

Nonmembers: All change of address information to be sent to subscriptions@medknow.com (i.e., not only for non-members).

**Advertising policies**

The journal accepts display and classified advertising. Frequency discounts and special positions are available. Inquiries about advertising should be sent to Wolters Kluwer India Private Limited, advertise@medknow.com.

The journal reserves the right to reject any advertisement considered unsuitable according to the set policies of the journal.

The appearance of advertising or product information in the various sections in the journal does not constitute an endorsement or approval by the journal and/or its publisher of the quality or value of the said product or of claims made for it by its manufacturer.

**Copyright**



**Permissions**

For information on how to request permissions to reproduce articles/information from this journal, please visit www.jnsmonline.org

**Disclaimer**

The information and opinions presented in the Journal reflect the views of the authors and not of the Journal or its Editorial Board or the Publisher. Publication does not constitute endorsement by the journal. Neither the Journal of Nature and Science of Medicine nor its publishers nor anyone else involved in creating, producing or delivering the Journal of Nature and Science of Medicine or the materials contained therein, assumes any liability or responsibility for the accuracy, completeness, or usefulness of any information provided in the Journal of Nature and Science of Medicine, nor shall they be liable for any direct, indirect, incidental, special, consequential or punitive damages arising out of the use of the Journal of Nature and Science of Medicine. The Journal of Nature and Science of Medicine, nor its publishers, nor any other party involved in the preparation of material contained in the Journal of Nature and Science of Medicine represents or warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for the results obtained from the use of such material. Readers are encouraged to confirm the information contained herein with other sources.

**Addresses**

*Editorial Correspondence*

**Prof. Ahmed BaHammam FRCP, FCCP,**
Professor of Medicine Consultant Pulmonary and Sleep Medicine Director,
University Sleep Disorders Center King Saud University, Saudi Arabia.
E-mail: jnsm@ksu.edu.sa; ashammam@ksu.edu.sa

**Published by**
**Wolters Kluwer India Private Limited**
A-202, 2nd Floor, The Qube, C.T.S. No.1498A/2
Village Marol, Andheri (East), Mumbai - 400 059, India.
Phone: 91-22-66491818
Website: www.medknow.com
*Printed at:*
Dhote Offset Technokrafts Pvt. Ltd. Goregaon (E), Mumbai, India

# Journal of Nature and Science of Medicine

**Official Publication of the College of Medicine, King Saud University**

Volume 1 ¦ Issue 2 ¦ July-December 2018

# Contents

Review Article

# Pharmacognostical, Phytochemical and Pharmacological Aspects of *Quisqualis indica*: An Update

**Mayank Kulshreshtha, Karuna Shanker Shukla[1], Garima Awasthi Tiwari[1], Manjul Pratap Singh[2], Anita Singh[3]**

Departments of Pharmacology, [1]Pharmaceutical Chemistry and [2]Pharmaceutics, School of Pharmacy, Babu Banarasi Das University, Lucknow, [3]Department of Pharmaceutics, Sunderdeep Pharmacy College, Ghaziabad, Uttar Pradesh, India

## Abstract

Nature is a big and important source of lots of things, in which plants are the living gifts of nature. Plants play an important role in various fields, but the role of these plants as herbal medicines is a big achievement. Drugs are obtained from plants have very low side effects and very popular in worldwide. Here, this review represents the pharmacology, pharmacognosy, and various molecular aspects of *Quisqualis indica* which may be helpful in near future based on available published data. This review opens the various doors or acts as a backbone for various researches in near future.

**Keywords:** Pharmacognosy, pharmacology, *Quisqualis indica*

## Introduction

Medicinal plants are defining various types of plants which have a capability to cure different diseases due to the presence of lots of primary and secondary metabolites. Such plants act as a backbone of traditional medicine.[1] These plants play an important role in various research, development of formulations, prepare the monograph, etc.[2] Herbal medicines are also known as botanical medicines or phytomedicines because different parts of plants using various purposes.[3] On behalf of Sumerian clay slab from Nagpur (approximately 5000 years old), the medicinal plants must needed for the preparation of medicines.[4] The Chinese book on roots and grasses "Pen T'Sao," written by Emperor Shen Nung circa 2500 BC, treats 365 drugs (dried parts of medicinal plants), many of which are used even nowadays such as the following: Rhei rhizoma, camphor, Theae folium, *Podophyllum*, the great yellow gentian, ginseng, jimson weed, cinnamon bark, ephedra, etc.[5,6] The Indian holy books Vedas mention treatment with plants, which are abundant in that country. Numerous spice plants used even today originate from India: Nutmeg, pepper, clove etc.[7] According to the World Health Organization, 80% of people from developing countries are depend on traditional system of medicine for curing the disease at beginning whereas in Indian materia medica includes about 2000 drugs of natural origin almost all of which are derived from different traditional systems and folklore practices. Out of these drugs derived from traditional system, 400 are of mineral and animal origin while the rest are of the vegetable origin. India has a rich heritage of traditional medicine and the traditional health care system has been flourishing in many countries.[8] It is also famous as "Botanical garden of world" whereas medicinal herbs are used before thousands of years in one form or another. Global estimates clearly proved that over 3/4$^{th}$ of the 5 billion world population cannot afford the products of the Western pharmaceutical industry and have to depend on traditional medicines from plants source.[9,10] On behalf of literature survey, it is concluded that there is a lack of complete scientific data available on *Quisqualis indica (Q. indica)* so that this compiled data may serve as a supporting reference for future research work, anatomy and physiology of the plant, formulation development, preparing monograph and may act as a backbone to cure various diseases in future.

| Access this article online | |
|---|---|
| Quick Response Code: | Website: www.jnsmonline.org |
| | DOI: 10.4103/JNSM.JNSM_20_18 |

**Address for correspondence:** Prof. Mayank Kulshreshtha, Department of Pharmacology, School of Pharmacy, Babu Banarasi Das University, Babu Banarasi Das City, Faizabad Road, Chinhat, Lucknow - 227 105, Uttar Pradesh, India.
E-mail: professormayank@gmail.com



**How to cite this article:** Kulshreshtha M, Shukla KS, Tiwari GA, Singh MP, Singh A. Pharmacognostical, phytochemical and pharmacological aspects of *Quisqualis indica*: An update. J Nat Sci Med 2018;1:41-7.

*Q. indica* Linn, family - Combretaceae, is a strong climber, ligneous vine that can reach from 2.5 meters to up to 8 meters. It is commonly known as Rangoon creeper. It is indigenous in Africa, Indo Malaysian region and cultivated all over India.[11] It is vining and evergreen plant which is having vigorous growth needing sturdy support and can get quite out-of-hand on its favorable growing site; it does not require deep and anchoring roots.[12] It generally requires an area with full sunlight, regular watering to keep the soil moist, and need a support stand for the vine to grow on. For the proper growth of any plant, it should be provided with basic requirements having well-maintained conditioning, i.e., sunlight, water, and fertilizer.[13] It is a widely known garden climber; the scarlet Rangoon creeper is a native of Africa which was introduced in the tropics as a popular ornamental. Botanically known as *Q. indica*, the creeper can often be seen as a hedge plant or covering compound walls. Genus name *Quisqualis* is derived from the Malay name "Udani" and refers to the variable habit and coloring of the plant. Species name *indica* refers to being from India.[14,15]

## Plant Botany

Common Names – Rangoon Creeper, Drunken Sailor, Akar Dani, Akar Suloh, Dani, Ara Dani, Akar Pontianak, Red Jasmine.

Vernacular name – English: Rangoon Creeper; Hindi: Madhumalti; Marathi: Rangoonvel, Madhumalalati, Vilayati Chameli; Gujarathi: Barmasivel; Bengali: Malati, Modhumalati; Telugu: Radha Manoharam; Manipuri: Parijat.

### Scientific classification

- Kingdom: Plantae
- Order: Myrtales
- Family: Combretaceae
- Genus: *Combretum*
- Species: *Combretum indicum*
- Binomial name: *C. indicum* (L.) De Filipps
- Synonym: *Q. indica* L [Figure 1].

Plant propagation requirements; Light preference: Full Sun; Water preference: Moderate water; Plant growth rate: Fast; Propagation method: Seed, Stem cutting.[15-18]

## Cultivation and Collection

*Q. indica* requires full sunlight with regular watering, but in hot season, it requires more water. Few varieties of *Q. indica* are distinguished, showing variations in flower color and leaf size. The plant is easily raised from layers, cuttings, or divisions of the root. It grows well in good soil. It grows rapidly, requiring a strong trellis for its support. It is also grown on an arch or on a tree. It can keep within bounds as a bush by removing the long new growths. The plant is in profuse blooming throughout the year. Flowers open in the evening as white flowers, gradually assuming pink tinge by morning and deepening to deep red by late afternoon. They are sweet-scented. Plant bears fruits in Northern India.[18]

**Figure 1:** *Quisqualis indica*

## Traditional Uses

*Q. indica* is cultivated as an ornamental plant due to its bright flowers with pleasant fragrance. It is used as ascariasis, ringworm disease, and infant malnutrition.[19] Seeds decoction in oil is applied topically in skin diseases. Seeds are the source of fatty oil which is purgative in action.[20] *Q. indica* (Fruits and seeds) are reported to have anthelmintic potential due to the presence of an active principle resembling Santorin. Seeds are found to cause colic in some cases and possess soporific properties. An overdose causes unconsciousness. Roasted ripe seeds are and given in diarrhea, fever, and in case of rickets in China. Seeds are also useful in skin diseases. The extracts of roots and leaves are also effective as anthelmintic. Leaf juice is used by Malays as a lotion for boils and ulcers. Long stems are used for basketry, fish wires, and fish traps in Togoland. Roots are used to treat rheumatism, also can be used to expel parasitic worms or for alleviating diarrhea.[21] In Amboyna, the leaves are given in compound decoction for flatulent distension of the abdomen.[13] It is also made into pills or powder. For adults, the cooked herb is chewed, with 10-20 pieces taken for one dose. For children, the number of pieces to be taken is 1.5 multiplied by the child's age, with the total number not to exceed 20 pieces/day.[22]

## Pharmacognostic Parameters of *Q. indica*

### Morphological characters

Flowers are fragrant, tubular, showy, first white, then becoming red, reddish-purple or orange, exhibiting the range of colors in clusters, on the same flower stalk.[23] Eisikowitch and Rotem justified the near about 3 days are reported to have flowering period of *Q. indica*. In the night, the flowers are treated by hawk moths while flowers are red color completely avoided by night visitors. In a day both types of flowers are treated by solitary bees, honeybees, flies, and sunbirds. Pollen grains germinate well on the stigmatic fluid during the first few hours, but germination is decreases during the day. Pollen tubes do not penetrate into the style, and seeds are not produced in Israel.

Nectar flow begins at flower dehiscence, reaches its peak at early morning, and then is absorbed by the flower. During the 1st h of blooming, the flower is typically "hawk moth" but, by the next morning, attracts visitors other than hawk moths.[24] Fruit is narrowly ellipsoid, 2.5–3 cm long, with five, sharp, longitudinal angles, or wings. Seeds are pentagonal and black.[23]

Fresh leaves of *Q. indica* are dark green color, compound, alternate arrangement, 8–10 pairs of veins are present, slightly crenate to entire margin, acuminate apex, ventral surface is smooth, dorsal surface is rough, pinnate venation, base symmetrical ovate shaped, bitter taste and odorless.[25]

### Microscopical characters and powder analysis of leaves

Mayank *et al*. studied the microscopic characters of leaf-like upper epidermis, lower epidermis, parenchymatous cells, colenchymatous cells trichomes, xylem, and phloem. Whole of the midrib filled with collenchymas with different types of trichomes such as covering and glandular. Midrib is almost triangular shows the presence of endodermal layer; it is a single layered, surrounds with vascular bundle, packed with starch grains. Endodermis covers vascular bundle and contains a number of starch grains. Leaf surface (upper and lower surface) study shows the presence of epidermal cells, paracytic stomata, and subsidiary cells. Powder microscopy proved the presence of vessels, covering trichome, glandular trichome calcium oxalate crystals, epidermal cells, paracytic stomata.[25]

### Phytochemical screening, physiochemical analysis, and quantitative microscopy

Various primary and secondary metabolites reported in different extracts of *Q. indica* are shown in Table 1. Physiochemical analysis and quantitative microscopy of leaves are shown in Tables 2 and 3.[25-28]

## Pharmacological Investigation and Molecular Research

### Antimicrobial activity

Mukherjee and Chandra investigated the antimicrobial activity of petroleum ether extract of *Q. indica* flowers using agar well-diffusion method against pathogenic bacteria *Staphylococcus aureus (S. aureus), Escherichia coli (E. coli), Pseudomonas aeruginosa (P. aeruginosa), Bacillus subtilis (B. subtilis)* and showed best antimicrobial activity. MIC values were 27.0, 30.0, 38.0, and 40.0 μg/ml for *S. aureus*, *E. coli*, *P. aeruginosa*, and *B. subtilis* respectively.[29] Mayank *et al*. proved the antimicrobial activity of *Q. indica* leaves against *S. aureus*, *E. coli*, *P. aeruginosa*, *B. subtilis* and *Aspergillus tubingensis* where it was found that leaves extract (aqueous and ethanolic) were active against all species but least affected with *E. coli*.[30] Fatima and team were investigating the antimicrobial potential of *Q. indica* bark against various microbial species, and it was found to be good antimicrobial property.[31] All parts of this plant have good antimicrobial property, but leaves were found to be better one.

### Antiviral activity

Antiviral activities of *Q. indica* leaf extracts on three selected avian viruses were evaluated. The assay was performed in 10-day-old embryonated chicken eggs by chorioallantoic membrane and the allantoic sac inoculation for infectious bursal disease virus (IBDV) and new castle disease virus (NDV), respectively. The viral replication in the tests and controls was estimated by hemagglutination assay of harvested allantoic fluid for NDV and reduction in pocks formation when compared with controls as an indication of viral inhibition in feline panleukopenia virus (FPV) and IBDV. At a concentrations of 400 mg/ml, 200 mg/ml and 100 mg/ml *Q. indica* yielded a percentage inhibition of 50.0%, 50.0%, and 43.0% for aqueous extract; 86.0%, 50.0% and 50.0% for ethanol extract; and 94.6%, 90.5%, and 42.6% for methanol extraction, respectively, on NDV. The challenged virus FPV recorded no activity with *Q. indica* and hundred percentage (100%) egg mortality was observed at the end of the experiment with IBDV.[32]

### Control lungworm infection

Ida *et al*. studied the application of 10% extract of wudani leaf (*Q. indica* Linn) may decrease the potential of becoming embryos of eggs of *Fasciola gigantica* and *Paramphistomum* sp. worms under *in-vitro* evaluation and concluded infection of lungworm predominantly occurs in cattle, the present results may revealed the opportunity of applying the wudani leaf extract to control lungworm infection in cattle.[33]

### Anthelmintic activity

Anthelmintics activities of different leaf extracts of *Q. indica* were evaluated separately on adult Indian earthworm (*Pheretima posthuma*). It was found that methanolic extract and aqueous extract of *Q. indica* showed anti-helminthes activity at a concentration of 60 mg/ml of each.[34]

### Insecticidal activity

Methanolic and ethyl acetate extracts of *Q. indica* L. flowers were used for their antifeedant and insecticidal action against third in star larvae of *Spodoptera litura* under laboratory condition. The results revealed that antifeedant activity was significantly superior in crude methanol extract of *Q. indica* flowers.[35]

### Anti lymphatic filariasis activity

Chen was proposed that *Q. indica* in this study to be a source of drug candidates to treat lymphatic filariasis because it has been used against parasitic infections in traditional Chinese medicine for over 1700 years. Its aqueous extract (2.62, 2.94, and 3.24 mg/mL) was shown effective in eliminating parasites of all three life stages (L3, adult male, and adult female).[36]

### Silver nanocrystals of *Quisqualis indica* against malaria and zika virus mosquito vectors

Marimuthu *et al*. were focused on the biophysical properties and the mosquitocidal action of *Q. indica*-fabricated AgNPs. AgNPs were characterized using spectroscopic (UV, FTIR, and XRD) and microscopic (AFM, SEM, TEM,

**Table 1: Phytochemical analysis of different extracts of *Quisqualis indica***

| Phytoconstituents | AEQIL | EEQIL | MEQIL | AEQIF | MEQIF | PEQIF | HAEQIF | MEQIAP |
|---|---|---|---|---|---|---|---|---|
| Alkaloids | + | + | + | + | + | + | + | + |
| Glycosides | + | + | + | + | + | - | + | + |
| Tannins | + | + | + | - | - | - | + | + |
| Flavonoids | + | + | + | + | + | + | + | + |
| Fats and oil | + | + | + | - | - | - | + | - |
| Carbohydrates | + | + | + | - | - | - | + | - |
| Reducing sugar | + | + | + | - | - | - | + | - |
| Proteins | + | + | + | - | - | - | + | + |
| Saponin | - | - | + | + | + | - | + | + |
| Terpenoids | + | + | + | + | + | - | - | + |

AEQIL: Aqueous extract of *Quisqualis indica* leaves, MEQIL: Methanolic extract of *Quisqualis indica* leaves, EEQIL: Ethanolic extract of *Quisqualis indica* leaves, AEQIF: Aqueous extract of Quisqualis indica flowers, MEQIF: Methanolic extract of *Quisqualis indica* flowers, PEQIF: Petroleum ether extract of *Quisqualis indica* flowers, HAEQIF: Hydroalcoholic extract of *Quisqualis indica* flowers, MEQIAP: Methanolic extract of *Quisqualis indica* aerial parts +: Present of metabolites –: Absent of metabolites

**Table 2: Quantitative microscopy of leaf of *Quisqualis indica***

| Parameters | Results |
|---|---|
| Vein islet number (1 mm² leaf surface) | 26.4 |
| Vein termination number (1 mm² leaf surface) | 52.4 |
| Stomatal number (1 mm² leaf surface on lower epidermis) | 96.32 |
| Stomatal number (1 mm² leaf surface on upper epidermis) | 70.45 |
| Stomatal index | 23.8 |
| Palisade ratio | 4.36 |

**Table 3: Physiochemical analysis of leaves of *Quisqualis indica***

| Parameters | Results |
|---|---|
| Total ash | 7.84 |
| Water soluble ash | 3.4 |
| Acid-insoluble ash | 1.2 |
| Water extractive value | 60.1 |
| Ethanol extractive value | 19.9 |
| Loss on drying | 7.8 |
| Swelling index | 2.4 |
| Foaming index | <100 |

and EDX) techniques. AFM, SEM, and TEM confirmed the synthesis of poly-dispersed AgNPs with spherical shape and size ranging from 1 to 30 nm. XRD shed light on the crystalline structure of these AgNPs and concluded that the proposed one-pot biogenic fabrication of AgNPs using *Q. indica* is a low-cost and eco-friendly tool in the fight against zika virus, malaria, and filariasis vectors, with little impact against non-target aquatic mosquito predators.[37]

### Against the inflammation of esophagus

In this study, effect of ethanolic flower extract of *Q. indica* on experimental esophagitis in albino Wister rats at 100, 200, and 300 mg/kg were subjected to pylorus and forestomach ligation. Results revealed that treatments with pantoprazole and flower extracts significantly inhibited the gastric secretion, total acidity, and esophagitis index.[38]

### Analgesic activity

The aim of the present study was designed to evaluate the analgesic activity of hydroalcoholic extract of *Q. indica* Linn. leaves in Wistar rats at 100 and 200 mg/kg p.o. and concluded that the hydroalcolic extract possessed dose-dependent, significant ($P < 0.05$) analgesic activity against experimentally induced pain.[39]

### Antioxidant activity

This study focused on the sequential extraction from *Q. indica* leaves using four different solvents (petroleum ether, chloroform, methanol, and water); however, the help of using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, reducing power assay, total antioxidant activity, and reduction of ferric ions and aqueous extract showed highest DPPH radical scavenging activity compared to other extracts, while chloroform extract demonstrated strongest antioxidant activity for rest of the three methods. Petroleum ether and methanol extract exhibited moderate antioxidant property.[40]

### Anti-inflammatory activity

Yashraj *et al*. evaluated the anti-inflammatory activity of hydroalcoholic extract of *Q. indica* in Wistar rats and established some pharmacological evidence to support the folklore claim that *Q. indica* L. is used as anti-inflammatory agent.[41]

### Antidiarrheal activity

Petroleum ether extract of leaves of *Q. indica* L. used against experimentally induced diarrhoea at the doses (100, 200 mg/kg, p.o) and concluded that possessed dose-dependent, significant ($P < 0.05$) antidiarrheal activity.[42]

### Acetylcholinesterase inhibition

In the search for new acetylcholinesterase inhibitors from plant origin, it was demonstrated that methanolic extract of *Q. indica* flower exhibited this activity. The extract inhibited electric acetylcholinesterase in dose-dependent manner with an $IC_{50}$

value of 0.77 μg/ml. The Michaelis–Menton constant (Km) for the hydrolysis of acetylcholine iodide was 0.034 mM. The Km value in the presence of the extracts (Kmapp) at first decreased, and then increased by 60%–88.9%. The Vmax was 0.017 μM/min/μg protein. The Vmax value in the presence of the extracts (Vmaxapp) decreased by 2.8%–52.3%. The estimated value of KI was 1.41 mM, respectively.[43]

### In case of hyperlipidemia

The effect of two different doses (100 mg/kg and 200 mg/kg) of ethanolic extract of aerial part of *Q. indica* on cholesterol diet (coconut, biscuit, and milk powder) induced hyperlipidemia was investigated in rats and concluded that methanolic extract was more effective than aqueous extract but at the dose of 200 mg/kg of methanolic extract had markly showed effects comparable antihyperlipidemic as that of standard atorvastatin.[44]

### Anticancer activity

In this study, 25-O-acetyl-23, 24-dihydro-cucurbitacin F identified as a cytotoxic constituent from *Q. indica* fractionated by chromatographic techniques and elucidated the chemical structures by Nuclear Magnetic Resonance and Mass spectrometry (MS). The $IC_{50}$ values for miltirone of 60 National Cancer Institute cell lines were associated with the microarray-based expression of 9706 genes.[45]

### Antidiabetic activity

Bairagi *et al*. investigated the antidiabetic potential of *Q. indica* flowers using alloxan-induced diabetes models at doses of 100, 200, and 400 mg/kg, p.o. for 43 days and concluded that plant extract significantly decreased the blood sugar level.[46]

### Immunomodulatory activity

Yashraj *et al*. proved the immunomodulatory activity of *Q. indica* flowers using carbon clearances test, cyclophosphamide-induced myelosuppression models at the dose of 100 and 150 mg/kg and concluded that higher dose showed significant immuomodulatory activity. Levamisol (50 mg/kg, p.o.) was used as standard drug. Cyclophosphamide (50 mg/kg) was used to induce myelosuppression.[47]

### Improves benign prostatic hyperplasia

Ub Wijerathne *et al*. investigated therapeutic efficacy of *Q. indica* extract on treating benign prostatic hyperplasia (BPH) in LNCaP human prostate cancer cell line and a testosterone-induced BPH rat model. LNCaP cells were treated with *Q. indica* plus testosterone propionate, and androgen receptor and prostate-specific antigen expression levels were assessed by Western blotting. Therefore, findings suggest that *Q. indica* at attenuates the BPH state in rats through antiproliferative and proapoptotic activities and might be useful in the clinical treatment of BPH.[48]

### Passive smoking-induced hyperlipidemia

Jyoti *et al*. investigated that hypolipidemic activity of methanolic extracts of aerial parts of *Q. indica* including flowers on passive smoking (PS) induced hyperlipidemia in rats had been evaluated. Hyperlipidemia was induced by PS in a closed chamber having one burning cigarettes inside it. The hypolipidemic activity was analyzed by reading the blood serum level in UV at 505 nm after treated with reagent present in auto span diagnostic kit. Dose of methanolic extracts of *Q. indica* had been prepared by using distilled water, i.e., 200 mg/kg p.o. and significantly reduce the harmful lipid layer in blood serum at varying concentration and dose-dependent manner which shows that the plant carries the hypolipidemic properties and concluded that the plants extracts recover the disorders in lipid metabolism noted in hyperlipidemic state.[49]

## Analytical Analysis

### High performance thin layer chromatography

Mayank *et al*. performed the high-performance thin layer chromatography (HPTLC) of leaves of *Q. indica* for urosolic acid and lupeol and found out the good concentration in extract and screened out various proteins through the western blotting technique. The screened proteins were found to be good antimicrobial potential.[25]

### Gas chromatography-mass spectrometry

Gas chromatography-MS was performed using aerial part with methanol, ethyl acetate and hexane extracts and found the various compounds as shown in Table 4.[50]

## Future Prospects

The plant *Q. indica* have various primary and secondary metabolites that are responsible to cure various acute and chronic diseases, so there is lack of various pharmacological

**Table 4: Phytochemicals detected in different extracts of *Quisqualis indica***

| Compounds | | |
|---|---|---|
| **Methanol extract** | **Ethyl acetate extract** | **Hexane extract** |
| Diethylphthalate | Dodecane | Pentadecane |
| Isobutyl-o-phthalate | Heptadecane | Farnesene |
| Methyl isohexadecanoate | Farnesene | 6-methyl octadecane |
| Methyl linolelaidate | Phytol | Heptadecane |
| Phytol | Octacosane | Methyl hexadecanoate |
| Methyl tetradecanoate, 12-Me | Trans squalene | Methyl palmitate |
| Hexadecatrienal | Pentatriacotane | Methyl linolate |
| Trans squalene | Gama-tocopherol | Phytol |
| 1-dotriacontanol | Tetratetracontane | Methyl stereate |
| Nerolidol isomer | Vitamin E acetate | Trans-squalene |
| Tocoferol | Stigmasterol | Pentatriacotane |
| Vitamin E acetate | Heptacosane-1-chloro | Gama-tocopherol |
| Stigmasterol | Unknown compound | Stigmasterol |
| Viridiforol | - | Heptacosane-1-chloro |
| Cycloartenyl acetate | - | Vitamin E acetate |

activities which are not reported yet. The new researchers may be easily plain for those activities for the best exploration of plant. Lots of phytochemicals are detected in various parts of *Q. indica*, they may be curable for diseases and open the new doors for various researchers in future.

## Conclusion

Nowadays, the human population depends on herbals to cure the diseases and to choose the herbal medicine for primary action just because of low side effects. *Q. indica* is a beautiful ornamental plant but has lots of pharmacological activity and found to be safe at various level of research. Pharmacognostical parameters of plant proved the anatomy and physiology where as HPTLC analysis clearly raveled the *Q. indica* is a good source of ursolic acid and lupeol which are responsible to cure various diseases such as hepatic, cardiovascular, and neurological problems. The plants have major qualities which act as backbone of various field of research, and it needs to be good exploration in society. HPTLC analysis clearly ravealed the *Q. indica* is a good source of urosolic acid and lupeol which are responsible to cure various diseases such as hepatic, cardiovascular, and neurological problems.

### Acknowledgment

The author would like to express my hearty thanks to BBD University and National Botanical Research Institute, Lucknow, for providing library facilities to access the journals and books.

### Financial support and sponsorship

Nil.

### Conflicts of interest

There are no conflicts of interest.

## References

1. Davidson HI. Ecological ethno botany: Stumbling toward new practices and paradigms. MASA J 2000;16:1-13.
2. UNESCO. Culture and Health, Orientation Texts – World Decade for Cultural Development 1988 – 1997, Document CLT/DEC/PRO – 1996. Paris, France: UNESCO; 1996. p. 129.
3. Available from: https://www.omicsonline.org/natural-products/herbal-medicine-review-articles.php. [Last accessed on 2017 Feb 01].
4. Kelly K. The History of Medicine. New York: Facts on File; 2009. p. 29-50.
5. Bottcher H. Miracle Drugs. Zagreb: Zora; 1965. p. 23-139.
6. Wiart C. Etnopharmacology of Medicinal Plants. New Jersey: Humana Press; 2006. p. 1-50.
7. Tucakov J. Healing with Plants – Phytotherapy. Beograd: Culture; 1971. p. 180-90.
8. Mukerjee PK. Quality Control of Herbal Drugs. 1st ed. New Delhi: Business Horizons Publication; 2002. p. 2-24.
9. Chaudhri RD. Herbal Drugs Industry: A Practical approach to Industrial Pharmacognosy. 1st ed. New Delhi: Eastren Publisher; 2004. p. 1-5.
10. EIRI Books. Handbook of Medicinal and Aromatic Plants Cultivation, Utilization and Extraction Processes. Delhi: Published by Engineers India Research Institute; 2009. p. 1-3.
11. Joshi SG. Medicinal Plants. 1st ed. New Delhi: Oxford and IBH Publishing Co., Pvt.; 2002. p. 122-3.
12. Shih Chun TZ. "Stuartexchange" Niyog Niyogan. Art Guild for Education and Communication Foundation Inc.; 2011.
13. Kirtikar KR, Basu BD. Indian Medicinal Plant. 2nd ed. New Delhi: International Book Publishers; 1987. p. 86-7.
14. Available from: http://www.thehindu.com/thehindu/mag/2003/03/16/stories/2003031600300800.htm. [Last accessed on 2017 Feb 01].
15. Available from: https://www.florafaunaweb.nparks.gov.sg/special-pages/plant-detail.aspx?id=1497. [Last accessed on 2017 Feb 01].
16. Nadkarni AK. Indian Materia Medica. 3rd ed. Mumbai: Popular Prakashan; 2000. p. 1046-7.
17. Kirtikar KR, Basu BD. Indian Medicinal Plants. 2nd ed. Delhi: Periodical Expert Book Agency; 1993. p. 1037-8.
18. The Wealth of India. A Dictionary of Indian Raw Materials and Industrial Products: Ph-Re. Vol. 8. New Delhi: Council of Scientific & Industrial Research; 2005. p. 357-8.
19. Prajapati ND, Kumar U. Agro's Dictionary of Medicinal Plants. Jodhpur: Agrobios; 2005. p. 282.
20. Sudha P. Useful Indian Herbs an Ethnobotanical Handbook. Delhi: Biotech Books; 2008. p. 248.
21. Lim TK. Edible Medicinal and Non-Medicinal Plants. Spain: Springer Science; 2014. p. 698-700.
22. Jing-Nuan WU. An Illustrated Chinese Materia Medica. New York: Published by Oxford University Press; 2005. p. 542-3.
23. Available from: http://www.stuartxchange.com/Niyog.html. [Last accessed on 2017 Feb 01].
24. Eisikowitch D, Rotem R. Flower orientation and color change in *Quisqualis indica* and their possible role in pollinator partitioning. Bot Gaz 1987;148:175-9.
25. Mayank K, Gunja S, Manjul PS. Pharmacognostical, anti-oxidant activity and high performance thin layer chromatography studies on leaves of *Quisqualis indica linn*. Curr Tradit Med 2018. [Epub a head of print].
26. Paech D, Tracey MV. Modern Methods of Plant Analysis. Berlin: Springer-Verlag; 1995. p. 373-4.
27. Shamili G, Santhi G. Pharmacognostical standardaisation of the flowers of *Quisqualis indica*. World J Pharm Res 2017;6:1134-45.
28. Kaur CD, Saraf S. Development of photoprotective creams with antioxidant polyphenolic herbal extracts. Res J Med Plant 2012;6:83-91.
29. Mukherjee D, Chandra G. Flower extracts of *Quisqualis indica* as novel antibacterial agent against some pathogenic bacteria. Ann Pharmacol Pharm 2017;2:1040.
30. Kulshreshtha M, Dwivedi H, Singh MP. Antimicrobial effects of leaves of Indian herbal plants with reference to peptic ulcer. Environ Dis 2018;3:18-26.
31. Fatima NJ, Mohammad SR, Mahboob H, Mohammad AR. Antimicrobial activity and cytotoxicity of *Quisqualis indica*. OPEM 2008;8:53-8.
32. Onwuatuegwu JT, Abraham OJ, Umeoduagu ND. Determination of antiviral activities of *Quisqualis indica* leaf extracts on three selected Avian viruses. IDOSR JSTY 2017;2:19-32.
33. Ida BK, Made SA, Anak AG, Anak AN, Harya PD. Wudani leaf extracts (*Quisqualis indica* linn) astraditional medicine to control the incidence of cattle worm. Bali Med J 2017;6:17-22.
34. Sarma DS, Srinivasan R, Rajesh KD, Nagajyothi D, Prabhavathi V, Santhi BM. Evaluation of anthelminthic activity of leaves of *Quisqualis indica*. World J Pharm Pharm Sci 2015;4:819-24.
35. Anusree SS, Nisha MS, Sheela MS. Insecticidal activity of *Quisqualis indica* flower extract on *Spodoptera litura* fabricius. Int J Appl Pure Sci Agric 2016;2:98-103.
36. Chen N. Therapeutic Effects of *Quisqualis indica* on Lymphatic Filariasis. Theses, Dissertations, and Projects; 2014. p. 77-80. Available from: https://www.scholarworks.smith.edu/theses/40. [Last accessed on 2017 Feb 01].
37. Marimuthu G, Periasamy V, Shine K, Naiyf S, Alharbib GB. One-pot biogenic fabrication of silver nanocrystals using *Quisqualis indica*: Effectiveness on malaria and Zika virus mosquito vectors, and impact on non-target aquatic organisms. J Photochem Photobiol B Biol 2016;162:646-55.
38. Sarita S, Amit R, Siddhartha M, Srimanta S, Sutanu M, Sudipta S. Effect of ethanolic extract of *Quisqualis indica* L. flower on experimental esophagitis in albino Wistar rats. Indian J Exp Biol 2017;55:122-6.
39. Kavita K, Hemlata B, Avantika A. Analgesic activity of *Quisqualis*

*indica*. Pharm Chem J 2017;4:1-8.
40. Zahidul I, Mamun S, Foysal H, Sanjoy KM, Mir SA, Tanvir H. Phytochemical and biological studies of the *Quisqualis indica* leaves extracts. J Noakhali Sci Technol Univ 2017;1:9-17.
41. Yashraj Y, Mohanty PK, Kasture SB. Anti-inflammatory activity of hydroalcoholic extract of *Quisqualis indica* linn. flower in rats. Int J Pharm Life Sci 2011;2:977-81.
42. Nitu S, Govind M, Rajesh KS, Gnaneshwari D. Evaluation of anti-diarrheal activity of *Quisqualis indica* L. leaves. Indian J Nat Prod Resour 2013;4:155-60.
43. Penpan W Chutima L, Thawatchai P, Sindhchai K. Kinetics of acetylcholinesterase inhibition of *Quisqualis indica* linn. flower extract. Silpakorn Univ Sci Technol J 2007;1:20-8.
44. Jyoti S, Pushpendra KP, Balkrishna D. Effect of *Quisqualis indica* (linn) on cholesterol diet induced hyperlipidemia in wistar albino rats. Int J Pharm Res Dev 2012;4:86-94.
45. Efferth T, Kahl S, Paulus K, Adams M, Rauh R, Boechzelt H, *et al*. Phytochemistry and pharmacogenomics of natural products derived from traditional Chinese medicine and Chinese materia medica with activity against tumor cells. Mol Cancer Ther 2008;7:152-61.
46. Bairagi VA, Sadu N, Senthilkumar KL, Ahir Y. Anti-diabetic potential of *Quisqualis indica* linn in rats. Int J Pharm Phytopharmacol Res 2012;1:166-71.
47. Yashraj Y, Mohanty PK, Kasture SB. Evaluation of immunomodulatory activity of hydroalcoholic extract of *Quisqualis indica* linn. flower in wistar rats. Int J Pharm Life Sci 2011;2:686-9.
48. Ub Wijerathne C, Park HS, Jeong HY, Song JW, Moon OS, Seo YW, *et al*. *Quisqualis indica* improves benign prostatic hyperplasia by regulating prostate cell proliferation and apoptosis. Biol Pharm Bull 2017;40:2125-33.
49. Jyoti S, Pushpendra KP, Balkrishna D. Effects of methanolic extracts of *Quisqualis indica* (Aerial Parts) on passive smoking induced hyperlipidemia in rats. J Pharm Technol 2013;3:26-9.
50. Marimuthu G, Periasamy V, Shine K, Naiyf S, Alharbib GB. One-pot biogenic fabrication of silver nanocrystals using *Quisqualis indica*: Effectiveness on malaria and Zika virus mosquito vectors, and impact on non-target aquatic organisms. J Photochem Photobiol B Biol 2016;162:646-55.

Original Article

# Which Stem Cells to Choose for Regenerative Medicine Application: Bone Marrow and Adipose Tissue Stromal Stem Cells – Similarities and Differences

**Nehad M. Alajez[1], Dalia Al-Ali[1,2], Radhakrishnan Vishnubalaji[1], Muthurangan Manikandan[1], Musaad Alfayez[1], Moustapha Kassem[1,2,3], Abdullah Aldahmash[1,4]**

[1]Department of Anatomy, Stem Cell Unit, College of Medicine, King Saud University, [4]Prince Naif Health Research Center, King Saud University, Riyadh, Kingdom of Saudi Arabia, [2]Department of Endocrinology, Molecular Endocrinology Unit, University Hospital of Odense, University of Southern Denmark, Odense, [3]Department of Cellular and Molecular Medicine, Danish Stem Cell Center (DanStem), University of Copenhagen, Copenhagen, Denmark

## Abstract

**Background:** Clinical use of stromal stem cells in regenerative medicine is increasingly recognized as a promising treatment modality for age-related degenerative diseases based on the promising initial results of clinical trials. However, the magnitude of positive effects observed in these trials has been variable which can be explained by the lack of standardization of the stem cell products "cell product." Bone marrow-derived stromal (also known mesenchymal) stem cells (BM-hMSC) and adipose tissue-hMSC (AD-hMSC) have been used interchangeably in clinical trials employing stromal stem cells as they were thought to be functionally identical. **Methods:** In the present study, we performed an extensive side-by-side comparison of BM-hMSC and AD-hMSC for their CD marker expression using FACS analysis, molecular phenotype using global mRNA gene expression analysis, and functional studies for their *in vitro* differentiation capacity to osteoblasts and adipocytes. **Results:** We observed both stromal cell populations were CD44+ CD13+ CD90+ CD29+ CD105+ CD14− HLDR−. We also observed that they express common genetic signature consisting of 13,667 genes with enrichment in a number of pathways relevant to stem cell biology, for example, focal adhesion, insulin signaling, and mitogen-activated protein kinase signaling. On the other hand, we observed significant differences in their molecular phenotype with 3282 and 1409 genes differentially expression in BM-hMSC and AD-hMSC, respectively. Further analysis revealed higher expression of genes associated with osteoblast differentiation in BM-hMSC and those of adipocyte differentiation in AD-hMSC which correlated with their differential capacity for osteoblast versus adipocyte differentiation, respectively. **Conclusion:** Our data suggest that the clinical use of MSC in therapy depend on MSC site of origin, and thus, BM-hMSC are better suited for clinical trials aiming at enhancing bone regeneration. We suggest that molecular phenotype of stem cells is relevant approach for stem cell screening before their clinical transplantation.

**Keywords:** Adipose tissue, bone marrow, mesenchymal stromal cells, osteogenesis, pathways

## Introduction

Human stromal stem cells (commonly known as mesenchymal stem cells) (hMSCs) are adult multipotent stem cells that have the ability to differentiate into multiple mesodermal lineage cells, such as adipocytes, osteoblasts, and chondrocytes.[1,2] MSCs are being introduced into a number of clinical trials for tissue repair, for example, bone and cartilage defects, and for the enhancement of tissue regeneration, for example, heart following myocardial infarction, brain following stroke, or immune modulation for example, graft-versus-host disease (GvHD).[3] The standard site for obtaining human stromal cells is bone marrow (BM-hMSC) where the cells are located on the abluminal surface of blood vessels.[4] However, obtaining sufficient samples to derived sufficient number of cells required for clinical studies is a major limitation for wide spread use of BM-hMSC. Over last several years, MSC-like populations have been obtained from a wide range of tissues, for example, adipose tissue,[5] skin,[6] umbilical cord blood,[7] and placenta.[8] Among all these tissues, adipose tissue is an attractive choice to obtain cells needed for clinical studies due

Access this article online

Quick Response Code:

Website: www.jnsmonline.org

DOI: 10.4103/JNSM.JNSM_18_18

**Address for correspondence:** Dr. Nehad M. Alajez, Department of Anatomy, Stem Cell Unit, College of Medicine, King Saud University, Riyadh 11461, Kingdom of Saudi Arabia. E-mail: nalajez@gmail.com



**How to cite this article:** Alajez NM, Al-Ali D, Vishnubalaji R, Manikandan M, Alfayez M, Kassem M, *et al*. Which stem cells to choose for regenerative medicine application: Bone marrow and adipose tissue stromal stem cells – Similarities and differences. J Nat Sci Med 2018;1:48-54.

to the ease of obtaining samples, during operative procedure, for example, liposuction. Human adipose-derived stromal stem cells (AD-hMSC) have been reported to exhibit a similar phenotype to that of BM-hMSC and have been suggested as an alternative source for obtaining MSC for clinical trials.[9] However, a detailed analysis of the similarities and differences of these different cell populations at the molecular level has not clearly been defined.

The aim of the present study was to compare stromal cell populations obtained from human bone marrow and from human adipose tissue in terms of their phenotype, molecular profile, and their differentiation potential into osteoblasts and adipocytes.

## Materials and Methods

### Cells

Bone marrow-derived stromal (mesenchymal) stem cells were purchased from thermo fisher scientific (Thermo Fisher Scientific Life Sciences (Waltham, MA, USA). Adipose-derived mesenchymal stromal cells were isolated as described before.[5]

### Ethics statement

The use of human specimens in the current study was approved by the Institutional Review Board at King Saud University College of Medicine (10-2815-IRB).

### Cell culture

Cells were cultured in a basal culture medium of Dulbecco's Modified Eagle's medium (DMEM), supplemented with 4500 mg/L D-glucose, 4 mM L-glutamine, 110 mg/L 10% sodium pyruvate, 10% fetal bovine serum (FBS), 1% penicillin-streptomycin, and 1% nonessential amino acids. All reagents were purchased from Thermo Fisher Scientific Life Sciences (Waltham, MA, USA, http://www.thermofisher.com). Cells were incubated in 5% $CO_2$ incubators at 37°C and 95% humidity.

### Flow cytometry

Phenotype analysis was performed as previously described.[6] In brief, trypsinized cells were washed twice in phosphate-buffered saline (PBS) supplemented with 0.5% FBS and resuspended to a concentration of about $1 \times 10^5$ cells/antibody test. For direct immunofluorescence, 10 μl FITC-conjugated mouse anti-human CD34, CD90, CD45, CD13, CD3, PE-conjugated mouse anti-human CD146, CD73, CD29, HLA-DR, and APC-conjugated mouse anti-human CD105, CD14, and CD44 antibodies (BD Biosciences, USA) were used. Nonspecific signal was analyzed by using a FITC/PE/APC-conjugated mouse IgG1 isotype antibodies, respectively. Cells were analyzed using BD FACSCalibur flow cytometer (BD Biosciences) and events were gated in a dot plot of forward versus side scatter signals on linear scale. At least, 10,000 gated events were acquired on a log fluorescence scale and data were analyzed using Kaluza Software Version 1.2 (Beckman Coulter, Indianapolis, IN).

### Gene expression microarray

RNA isolation and gene expression analyses were carried out as described in our previously published manuscripts.[10] In brief, RNA was isolated using the total tissue RNA purification kit from Norgen Biotek Corp., (Thorold, ON, Canada) and was quantified using NanoDrop 2000 (Thermo Scientific, Wilmington, DE, USA). Total RNA was labeled and then hybridized to the Agilent Human SurePrint G3 Human GE 8 × 60 k microarray chip (Agilent Technologies, Santa Clara, CA, USA). All microarray experiments were conducted at the Microarray Core Facility (Stem Cell Unit, Department of Anatomy, King Saud University College of Medicine). Data were subsequently normalized and analyzed using GeneSpring 13.0 software (Agilent Technologies, Santa Clara, CA, USA). Pathway analyses were conducted using the Single Experiment Pathway analysis feature in GeneSpring 13.0 (Agilent Technologies).

### Adipogenic differentiation

The adipogenic induction medium (AIM) consisted of DMEM supplemented with 10% FBS, 10% horse serum (Sigma-Aldrich, St. Louis, MO, USA, http://www.sigmaaldrich.com), 1% penicillin/streptomycin, 100 nM dexamethasone, 0.45 mM isobutyl methyl xanthine (Sigma-Aldrich), 3 mg/mL insulin (Sigma-Aldrich), and 1 mM rosiglitazone (BRL49653). The AIM was replaced every 3 days. Cells were assessed for adipogenic differentiation on day 7.

### Oil Red O and Nile Red staining

Adipogenic differentiation was determined by qualitative Oil Red O staining for lipid-filled mature adipocytes. Cells were washed with PBS, fixed with 4% paraformaldehyde for 10 min, then incubated with freshly made and filtered (0.45 mM) Oil Red O staining solution (0.05 g in 60% isopropanol; Sigma-Aldrich) for 1 h at room temperature. Nile red fluorescence staining and quantification of adipogenesis was performed using a stock solution of Nile red (1 mg/mL) in DMSO that was stored at − 20°C and protected from light. Staining was performed on unfixed cells. Cultured differentiated cells were grown in polystyrene flat-bottom 96-well tissue culture-treated black microplates (Corning Inc., Corning, NY, USA, http://www.corning.com) and washed once with PBS. The dye was then added directly to the cells at a final concentration of 5 μg/mL in PBS, and the preparation was incubated for 10 min at room temperature, then washed twice with PBS. The fluorescent signal was measured using a SpectraMax/M5 fluorescence spectrophotometer plate reader (Molecular Devices Co., Sunnyvale, CA, USA, https://www.moleculardevices.com) using the bottom well scan mode, during which nine readings were taken per well using excitation (485 nm) and emission (572 nm) spectra. Oil Red and Nile red fluorescence was imaged using an EVOS Cell Imaging System (Thermo Fisher Scientific Life Sciences).

### Osteogenic differentiation

BM-hMSC were cultured as noted in the previous section and exposed to osteogenic induction medium

(DMEM containing 10% FBS, 1% penicillin-streptomycin, 50 mg/mL L-ascorbic acid [Wako Chemicals GmbH, Neuss, Germany, http://www.wakochemicals.de/], 10 mM β-glycerophosphate [Sigma-Aldrich], 10 nM calcitriol [1a, 25-dihydroxy vitamin D3; Sigma-Aldrich], and 100 nM dexamethasone [Sigma-Aldrich]).

### Alkaline phosphatase staining and activity quantification

We used a BioVision alkaline phosphatase (ALP) activity colorimetric assay kit (BioVision, Inc., Milpitas, CA, USA, http://www.biovision.com/) with some modifications.[11] Cells were cultured in 96 well plates under normal or osteogenic induction conditions. On day 10, wells were rinsed once with PBS and fixed using 3.7% formaldehyde in 90% ethanol for 30 s at room temperature. The fixative was removed, and 50 μL of p-nitrophenyl phosphate solution was added to each well. The plates were incubated for 20–30 min in the dark at room temperature until a clear yellow color was developed. The reaction was subsequently stopped by adding 20 μL of stop solution. Optical density was then measured at 405 nm using a SpectraMax/M5 fluorescence spectrophotometer plate reader. For ALP staining, the cells were washed in PBS, fixed in acetone/citrate buffer, and incubated with ALP substrate solution (naphthol AS-TR phosphate 0.1 M Tris buffer, pH 9.0) for 1 h at room temperature. Images were taken using an EVOS Cell Imaging System (Thermo Fisher Scientific Life Sciences).

### Statistics

Statistical analyses and graphing were performed using Microsoft excel 2010 and GraphPad Prism 6.0 software (GraphPad, San Diego, CA, USA). *P* values were calculated using a two-tailed *t*-test.

## Results

### Phenotypic characterization of bone marrow-derived human stromal stem cell and adipose-derived human stromal stem cell

A panel of surface markers was utilized to immunophenotype BM-MSC versus AD-MSC using FACS analysis [Figure 1a and b]. Both cell types were negative for the endothelial and hematopoietic lineage markers (CD34, CD45, CD14, CD31, and HLA-DR), whereas they were positive for the stromal cell-associated markers (CD13, CD29, CD44, CD73, CD90, and CD105). This CD markers' panel indicated that they were of nonhematopoietic or endothelial origin and expressed general stromal cells markers.

### Molecular profiling of bone marrow-derived human stromal stem cell and adipose-derived human stromal stem cell revealed common gene signature

Phenotypic data revealed similarities between BM-hMSC and AD-hMSC based on general surface markers; however, it is not clear whether the two cell populations exhibit similar molecular phenotype, i.e., molecular signature. Gene expression profiling of BM-hMSC versus AD-hMSC revealed clear separation of the two cell types [Figure 2a]. We observed similarities in gene expression between the two cell populations where a common signature consisting of 13,667 genes was expressed by the two cell populations [Figure 2b]. Pathway analysis of the common gene signature revealed enrichment in several

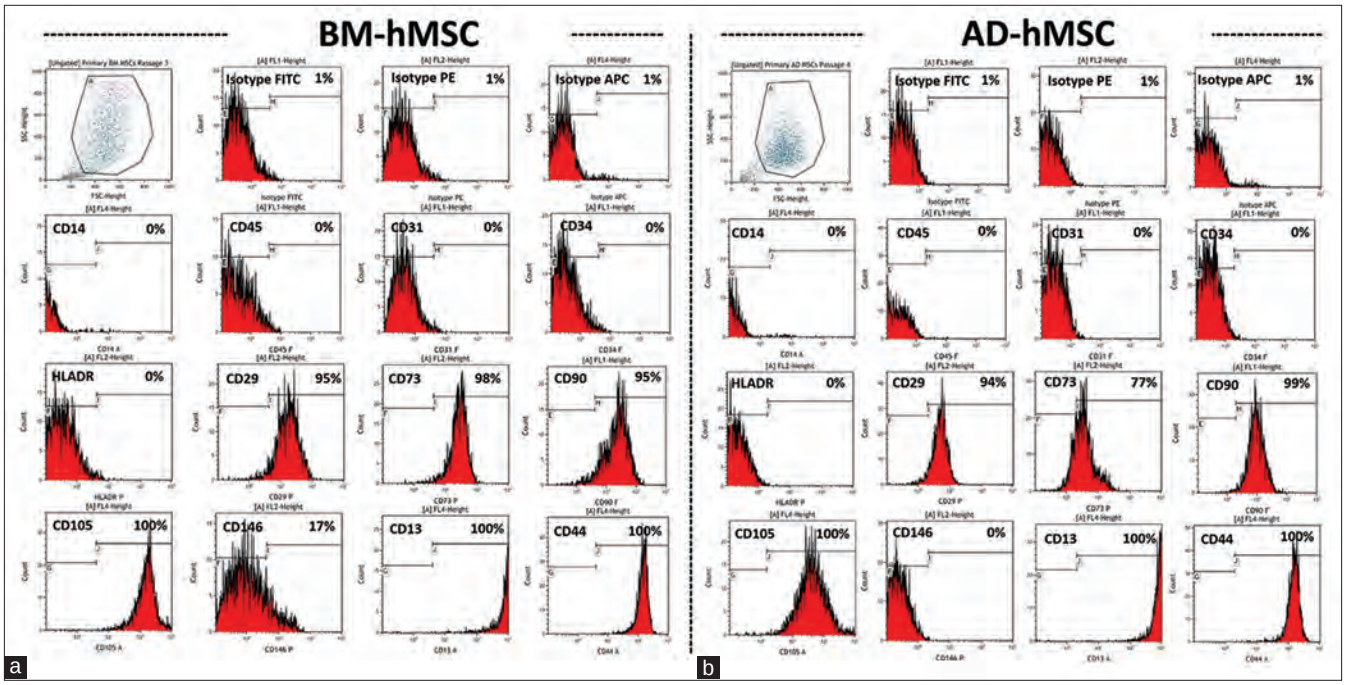


**Figure 1:** Flow cytometry analysis shows the phenotypic resemblance of BM-MSC and AD-MSC. Primary BM-MSC (a) and AD-MSC (b) were collected and were stained for the indicated surface markers and were analyzed by flow cytometry. The percentage of positive population is indicated on each plot. BM-MSC: Bone marrow-derived human stromal stem cell, AD-MSC: Adipose-derived human stromal stem cell

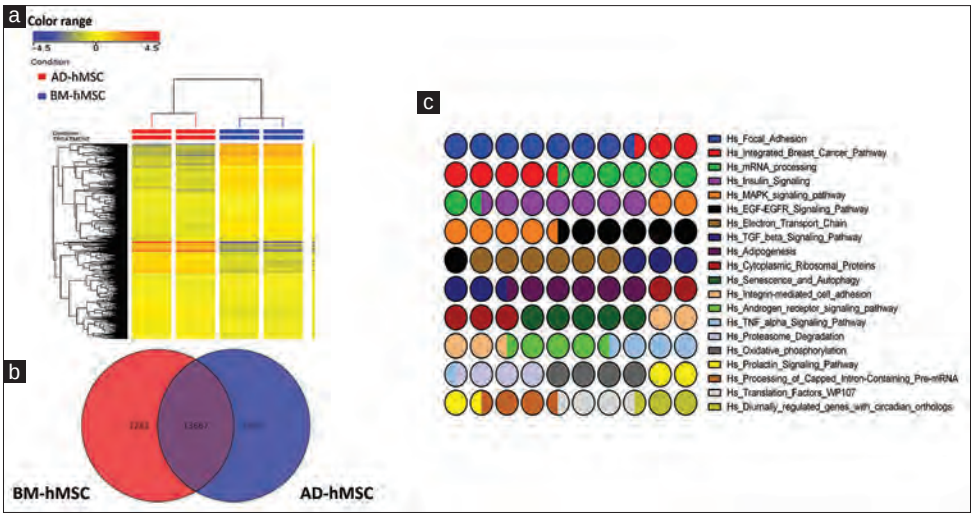


**Figure 2:** Microarray gene expression profiling of BM-MSC versus AD-MSC. (a) Heat map analysis and unsupervised hierarchical clustering were performed on expressed genes in BM-MSC versus AD-MSC. Each column represents one replica and each row represents a transcript. Expression level of each gene in a single sample is depicted according to the color scale. (b) Venn diagram illustrating the overlap between genes expressed in BM-MSC and AD-MSC. (c) Illustration of the top twenty enriched pathways performed on genes commonly expressed by BM-MSC and AD-MSC. BM-MSC: Bone marrow-derived human stromal stem cell, AD-MSC: Adipose-derived human stromal stem cell

pathways related to MSC biology such as focal adhesion, mitogen-activated protein kinase (MAPK), transforming growth factor beta (TGFb), and adipogenesis pathway. List of the top enriched pathways is illustrated in Figure 2c. Illustration of the FAK pathway with matched entities from the microarray data is shown in Figure 3.

### Bone marrow-derived human stromal stem cell is enriched in osteogenic genes while adipose-derived human stromal stem cell is more enriched in adipogenic genes

Although our data revealed high degree of similarities in gene expression profile of BM-hMSC and AD-hMSC, there existed significant differences [Figure 2b] where BM-hMSC differentially expressed 3282 genes whereas AD-MSC differentially expressed 1409 genes. Interestingly, when we compared the expression levels of a panel of osteogenic markers (BGLAP, DLX5, IGF2, TGFb1, TGFb2, and TGFBR2) as well as a panel of adipogenic markers (AdipoQ, CEBPA, CEBPB, FABP4, LPL, and PPARg) in both cell types, we observed higher expression of the osteogenic gene markers in BM-hMSC [Figure 4a] while the expression of adipogenic gene markers was higher in AD-hMSC [Figure 4b].

### Bone marrow-derived human stromal stem cell exhibits higher osteogenic while adipose-derived human stromal stem cell exhibits higher adipogenic differentiation potential

Based on gene expression data, we determined differences in the differentiation potential to osteoblasts and adipocytes between BM-hMSC and AD-hMSCs. Both cell populations were induced into osteoblastic cells, and on day 10, cells were stained for ALP activity. As shown in Figure 5a, higher levels of the osteoblastic marker ALP were observed in BM-hMSC compared to AD-hMSC. Concordantly, ALP enzymatic activity quantification revealed higher ALP activity in BM-hMSC compared to AD-hMSC [Figure 5b]. The adipogenic differentiation capacity of both cells types was also investigated. Enhanced adipogenic differentiation of AD-hMSC as compared to BM-hMSC based on quantification of mature adipocytes stained positive for Nile red was observed [Figure 5c and d].

## Discussion

There is an increasing interest in using stem cells in treatment of degenerative and age-related diseases, for example, Parkinson's disease, liver failure, leukemia, diabetes, osteoarthritis, and osteoporosis, for which there is no curative therapy. Furthermore, the need for novel approaches based on stem cell transplantation to enhance skeletal tissue regeneration and repair in cases of nonhealing fractures and bone defects is needed. The choice of functionally relevant cell type is important for the successful use of cells in therapy. Currently, bone marrow MSC and adipose tissue MSC were used interchangeably with the assumption that these cells are functionally similar. In the current manuscript, we demonstrate that these two cell population exhibit significant differences in their molecular phenotype and their functional differentiation capacity which is relevant to their clinical use.

The cellular phenotype of both BM-hMSC and AD-hMSC based on CD marker expression was similar suggesting that this cellular phenotype is characteristics of the stromal cell populations irrespective of their tissue of origin. Our data thus corroborate previous studies which exhibit similar panel of CD-surface markers known to be present in stromal cell populations.[5] Similar to the presence of a common CD markers signature, both BM-hMSC and AD-hMSC shared a large number of genes and enrichment in a number of genetic

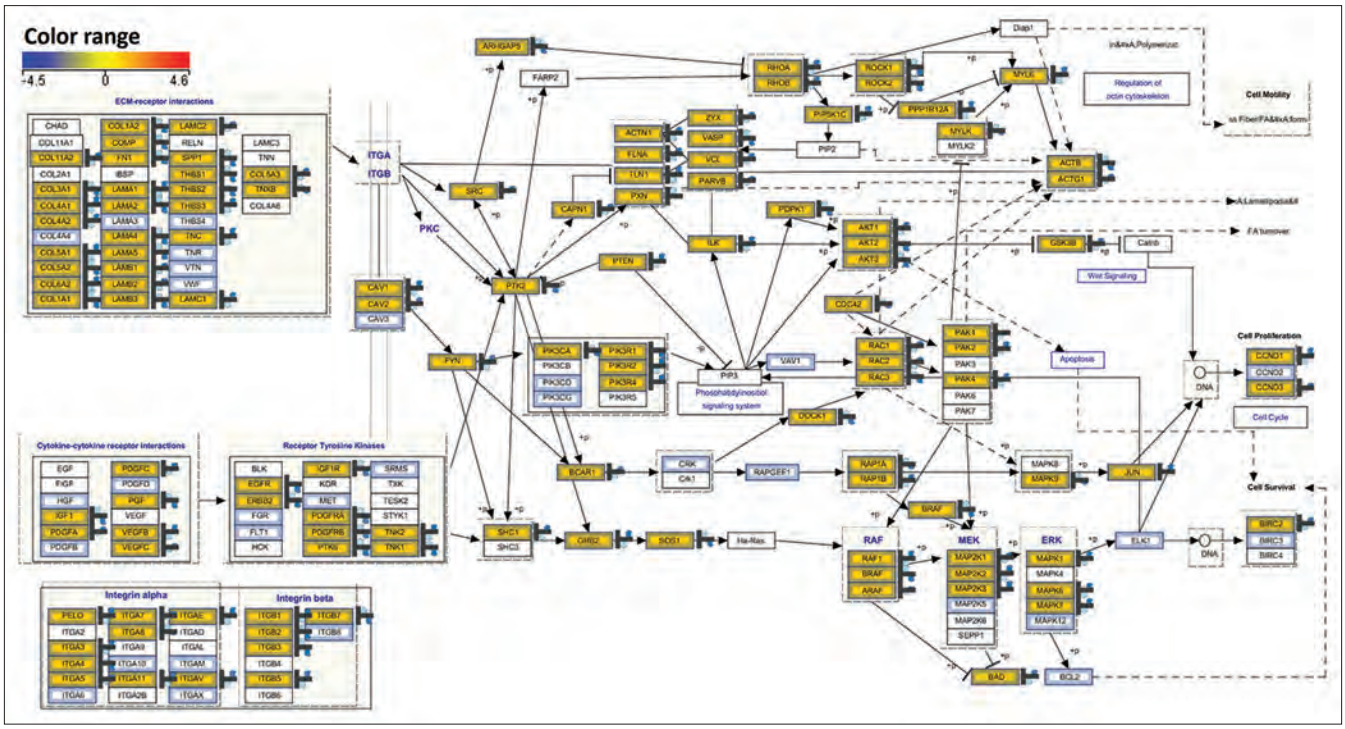


**Figure 3:** Illustration of the FAK pathway. Illustration of the FAK pathway enriched in the commonly expressed genes in BM-MSC and AD-MSC. Color scale indicates the expression level. Matched entities from the microarray data are highlighted. FAK: Focal adhesion kinase, BM-MSC: Bone marrow-derived human stromal stem cell, AD-MSC: Adipose-derived human stromal stem cell

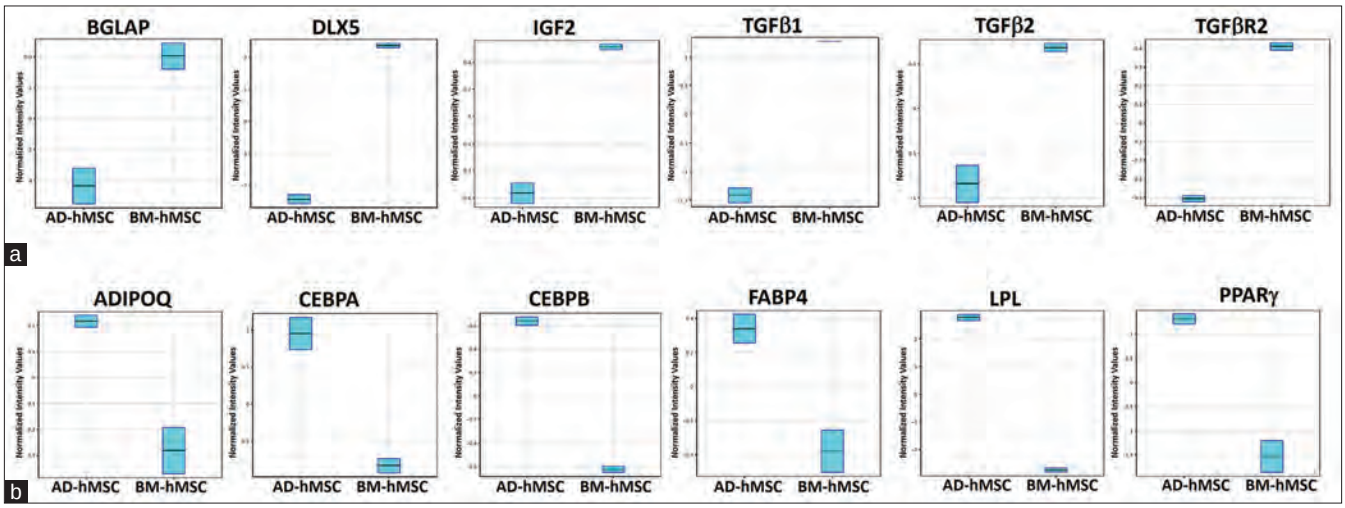


**Figure 4:** Expression of osteogenic and adipogenic gene markers in BM-MSC and AD-MSC. (a) Expression of a panel of osteogenic markers (BGLAP, DLX5, IGF2, TGFb1, TGFb2, and TGFBR2) in BM-MSC and AD-MSC based on microarray data. (b) Expression of a panel of adipogenic markers (AdipoQ, CEBPA, CEBPB, FABP4, LPL, and PPARg) in BM-MSC and AD-MSC based on microarray data. BM-MSC: Bone marrow-derived human stromal stem cell, AD-MSC: Adipose-derived human stromal stem cell

pathways that are required for stem cell function, for example, focal adhesion signaling, insulin signaling, and MAPK signaling. Their functions include self-renewal capacity of stem cells (MAPK), providing sufficient energy (insulin signaling) and cellular identity in their niche (Focal adhesion).

We observed also significant differences between BM-hMSC and AD-hMSC in terms of significantly enriched gene groups. Interestingly, BM-hMSC has higher expression of genes relevant to osteoblast differentiation while AD-hMSC has higher expression of genes relevant to adipocyte differentiation. Interestingly, genes that were unique to BM-hMSC were more enriched in cell cycle regulation, while that were unique to AD-hMSC were more enriched in immune modulation (data not shown). MSCs were first described as nonhematopoietic, plastic adherent, multipotent, mesodermal germ layer-derived cells by Friedenstein *et al*.[12]

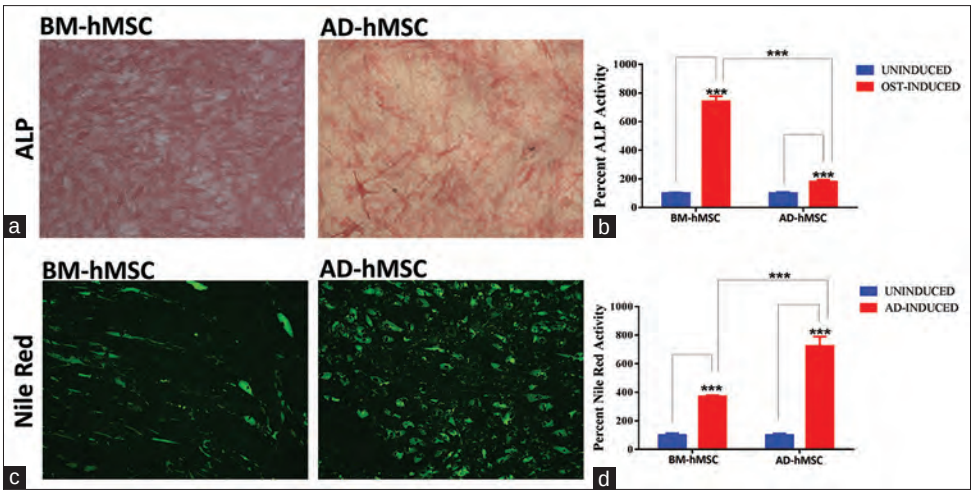


**Figure 5:** Differential osteoblastic and adipocytic differentiation of BM-MSC and AD-MSC. (a) Representative ALP staining on day 10 induction of BM-MSC (left panel) or AD-MSC (right panel). (b) Quantification of ALP activity in BM-MSC versus AD-MSC induced into osteoblast for 10 days. Data are presented as mean ± standard error of the mean; $n = 8$ from two independent experiments. ***$P < 0.0005$. (c) Representative Nile Red staining of lipid filled adipocytes on day 7 induction of BM-MSC (left panel) or AD-MSC (right panel). Images were captured using EVOS FL Auto system (Thermo) using ×10 objective. (d) Nile red quantification on day 7 after adipocytic induction of BM-MSC and AD-MSC. Data are presented as mean ± standard error of the mean; $n = 6$ from two independent experiments. ***$P < 0.0005$. BM-MSC: Bone marrow-derived human stromal stem cell, AD-MSC: Adipose-derived human stromal stem cell, ALP: Alkaline phosphatase

Interestingly, Friedenstein *et al*. termed bone marrow MSC as "committed osteoprogenitor" cells while MSC derived from other tissues as "inducible osteoprogenitor" based on their *in vivo* transplantation studies and the need of the "Inducible osteoprogenitor" cells for osteoblastic induction using growth factors, for example, bone morphogenetic proteins to reveal their osteogenic differentiation capacity. Our data provide molecular explanation for this phenomenon and demonstrate that AD-hMSC are poor are osteoblast differentiation compared to BM-hMSC.

We demonstrated that employing global gene expression of cultured cells, i.e., determining their molecular signature is predictive for their functional capacity. The molecular signature of BM-hMSC suggested commitment to osteoblastic differentiation and AD-hMSC suggested commitment to adipocytic differentiation which was confirmed in subsequent function studies. Our data may thus encourage using global gene expression analysis as an approach to determine the functional capacity of the cells before their use in clinical trials.[13]

## Conclusion

Our results have a clinical relevance as it demonstrates that "not all stem cells are equal" and thus proper choice of stem cells based on their expected functions following *in vivo* transplantation is needed. While the initial results of MSC used in clinical trials are promising, the magnitude of positive effects has been variable and likely caused by differences in the MSC populations employed as well as the lack of standardization of the MSC "cell product."[14] We suggest that in MSC cell-based therapy, it is important to employ well-characterized cell populations based on their molecular and functional phenotype that are aligned with the aim of their clinical use.

## Acknowledgment

We would like to thank the Deanship of Scientific Research at King Saud University (Research Group No. RG-1438-033).

## Financial support and sponsorship

This study was financially supported by Deanship of Scientific Research at King Saud University (Research Group No. RG-1438-033).

## Conflicts of interest

There are no conflicts of interest.

## References

1. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, *et al*. Multilineage potential of adult human mesenchymal stem cells. Science 1999;284:143-7.
2. Abdallah BM, Kassem M. Human mesenchymal stem cells: From basic biology to clinical applications. Gene Ther 2008;15:109-16.
3. Chamberlain G, Fox J, Ashton B, Middleton J. Concise review: Mesenchymal stem cells: Their phenotype, differentiation capacity, immunological features, and potential for homing. Stem Cells 2007;25:2739-49.
4. Sacchetti B, Funari A, Michienzi S, Di Cesare S, Piersanti S, Saggio I, *et al*. Self-renewing osteoprogenitors in bone marrow sinusoids can organize a hematopoietic microenvironment. Cell 2007;131:324-36.
5. Al-Nbaheen M, Vishnubalaji R, Ali D, Bouslimi A, Al-Jassir F, Megges M, *et al*. Human stromal (mesenchymal) stem cells from bone marrow, adipose tissue and skin exhibit differences in molecular phenotype and differentiation potential. Stem Cell Rev 2013;9:32-43.
6. Vishnubalaji R, Manikandan M, Al-Nbaheen M, Kadalmani B, Aldahmash A, Alajez NM, *et al*. *In vitro* differentiation of human skin-derived multipotent stromal cells into putative endothelial-like cells. BMC Dev Biol 2012;12:7.
7. Lee OK, Kuo TK, Chen WM, Lee KD, Hsieh SL, Chen TH, *et al*. Isolation of multipotent mesenchymal stem cells from umbilical cord blood. Blood 2004;103:1669-75.
8. Abumaree MH, Al Jumah MA, Kalionis B, Jawdat D, Al Khaldi A, AlTalabani AA, *et al*. Phenotypic and functional characterization of

mesenchymal stem cells from chorionic villi of human term placenta. Stem Cell Rev 2013;9:16-31.
9. De Ugarte DA, Morizono K, Elbarbary A, Alfonso Z, Zuk PA, Zhu M, *et al*. Comparison of multi-lineage cells from human adipose tissue and bone marrow. Cells Tissues Organs 2003;174:101-9.
10. Ali D, Abuelreich S, Alkeraishan N, Shwish NB, Hamam R, Kassem M, *et al*. Multiple intracellular signaling pathways orchestrate adipocytic differentiation of human bone marrow stromal stem cells. Biosci Rep 2018;38. pii: BSR20171252.
11. Ali D, Hamam R, Alfayez M, Kassem M, Aldahmash A, Alajez NM, *et al*. Epigenetic library screen identifies abexinostat as novel regulator of adipocytic and osteoblastic differentiation of human skeletal (Mesenchymal) stem cells. Stem Cells Transl Med 2016;5:1036-47.
12. Friedenstein AJ, Chailakhyan RK, Gerasimov UV. Bone marrow osteogenic stem cells: *In vitro* cultivation and transplantation in diffusion chambers. Cell Tissue Kinet 1987;20:263-72.
13. Larsen KH, Frederiksen CM, Burns JS, Abdallah BM, Kassem M. Identifying a molecular phenotype for bone marrow stromal cells with *in vivo* bone-forming capacity. J Bone Miner Res 2010;25:796-808.
14. Zaher W, Harkness L, Jafari A, Kassem M. An update of human mesenchymal stem cell biology and their clinical uses. Arch Toxicol 2014;88:1069-82.

Original Article

# The Role of Serum Levels of Thioredoxin and Thioredoxin-interacting Protein in Stroke

**Fawaz Al-Hussain, Muhammad Iqbal[1], Mohammed Al-Quwayee[1], Abdullah Bin Jurays[1], Muhannad Al-Wabel[1], Saqr Dayes[1], Fars Al-Manie[1], Tariq Al-Matrodi[1], Khalid Al-Regaiey[1], Shahid Bashir[2]**

Departments of Neurology and [1]Physiology, Faculty of Medicine, King Saud University, Riyadh, [2]Department of Neurophysiology, Neuroscience Center, King Fahad Specialist Hospital Dammam, Dammam, Saudi Arabia

## Abstract

**Aims**: Serum level of thioredoxin (TRX), a redox-regulating protein with antioxidant activity, increases under oxidative stress. The present study measured serum levels of TRX and its inhibitor TRX-interacting protein (TXNIP) in patients who experienced first-ever acute ischemic stroke (AIS). **Subjects and Methods:** We retrospectively enrolled 45 patients who experienced AIS and 33 age- and sex-matched healthy controls. Serum TRX and TXNIP levels in stroke patients and healthy controls were analyzed by performing solid-phase sandwich enzyme-linked immunosorbent assay. **Results**: Our results showed that mean serum TXNIP levels were significantly higher in stroke patients than in healthy controls ($P = 0.044$). However, serum TRX levels were not significantly different between stroke patients and healthy controls ($P = 0.405$). Moreover, we observed a significant positive correlation between TRX and TXNIP levels ($R^2 = 0.476$, $P < 0.003$). **Conclusions:** These results suggest that TRX and TXNIP are rapid, inexpensive, and convenient biomarkers of stroke. However, additional studies should be performed to validate these preliminary observations and the role of TRX and TXNIP in AIS.

**Keywords:** Ischemic stroke, thioredoxin, thioredoxin-interacting protein

## Introduction

Stroke is the third-leading cause of death worldwide and is a devastating endpoint of cerebrovascular diseases in many surviving patients.[1] Acquired brain injuries such as stroke continuously affect patients, families, and society because of the aging of the general population and the increasing length of postinsult survival. Data on stroke prevalence in Saudi Arabia are scarce. However, a recent study reported lower prevalence of stroke in Saudi Arabia than in Western and Asian countries and increased incidence of stroke in the younger population in Saudi Arabia.[2] Stroke is an important global health problem and a prime cause of death and disability worldwide.

Normal cellular metabolism produces low or moderate concentrations of reactive oxygen species (ROS) that participate in normal physiological processes. However, high concentrations of ROS are toxic to cellular components such DNA, proteins, and lipids, and cells cannot reverse the deleterious effects of ROS through antioxidant mechanisms.[3] This imbalance in the oxidant status of cells is called oxidative stress and is implicated in aging and development of cancer and other neurodegenerative diseases such as cancer and atherosclerosis.[4,5] Thioredoxin (TRX) system, which comprises NADPH, TRX reductase, and its substrate TRX, is an important antioxidant system.[6] The promoter of TRX contains a cis-regulatory region that is stimulated in the presence of oxidative stress induced by ischemic reperfusion, oxidative agents, or ultraviolet irradiation.[7]

TRX performs many biological functions, including ROS scavenging, thus exerting protective effects against oxidative stress; moreover, serum and plasma levels of TRX are good indicators of oxidative stress and are important biomarkers of different diseases.[8-11]

TRX-interacting protein (TXNIP) is an endogenous inhibitor of the TRX system. Overexpression of TRX in transgenic mice or knocking down TXNIP expression by siRNA exhibited

| Access this article online | |
|---|---|
| Quick Response Code: | Website: www.jnsmonline.org |
| | DOI: 10.4103/JNSM.JNSM_3_18 |

**Address for correspondence:** Dr. Shahid Bashir, Department of Neurophysiology, Neuroscience Center, King Fahad Specialist Hospital Dammam, Dammam, Saudi Arabia.
E-mail: sbashir10@gmail.com



**How to cite this article:** Al-Hussain F, Iqbal M, Al-Quwayee M, Jurays AB, Al-Wabel M, Dayes S, *et al*. The role of serum levels of thioredoxin and thioredoxin-interacting protein in stroke. J Nat Sci Med 2018;1:55-8.

neuroprotective effects in ischemic brain damage.[9,12] Increased TXNIP expression exerts proinflammatory and proapoptotic effects in stress-related diseases such as stroke.[13] Pharmacological inhibition of TXNIP prevented ischemic brain damage in an animal model by inhibiting oxidative stress and inflammasome activation.[14]

Due to the association between oxidative stress and stroke, we examined whether serum TRX and TXNIP levels were associated with the pathogenesis of stroke and could be used as reliable predictive markers for the diagnosis of stroke.

## Subjects and Methods

### Study population

We retrospectively analyzed 45 patients diagnosed with acute ischemic stroke (AIS), complete clinical data containing laboratory and magnetic resonance imaging records on admission were accessed. In addition, this study included 33 healthy controls without any risk factors or chronic diseases. The study was approved by our institute ethics committee.

### Blood collection and complete blood count analysis

Approximately 5 ml blood samples were collected from all the study participants in two tubes (2.5 ml blood sample in each tube) with and without ethylenediaminetetraacetic acid. Complete blood count was determined using Sysmex-XE 2000i automated blood cell analyzer (Sysmex, Kobe, Japan) within 1 h after collecting the blood samples. For performing biomarker analysis, serum was separated from the blood samples by performing centrifugation at 1500 ×g for 10 min, aliquoted, and stored at −80°C until further use.

### Biomarker assessment

Serum TRX and TXNIP levels were measured by performing sandwich enzyme-linked immunosorbent assay with human TRX and TXNIP enzyme-linked immunosorbent assay kits (Elabscience Biotechnology Co., Ltd., China), according to the manufacturer's instructions. In brief, precoated antibodies specific to TRX and TXNIP were incubated with samples, standards, and appropriate controls for 90 min at 37°C. After incubation, the samples were treated with biotinylated antibodies against TRX and TXNIP for 1 h at 37°C. After washing, antigen–antibody complexes were determined using an avidin–horseradish peroxidase conjugate. An enzyme substrate was used to hydrolyze the reaction, and the enzyme–substrate reaction was stopped by adding sulfuric solution. Intensity of the reaction was measured at 450 nm by ELISA microplate reader (BioTek Instruments, USA).

### Data analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences for Windows v20.0 (SPSS Inc., Chicago, IL, USA). Kolmogorov–Smirnov test was used to examine the normal distribution of data. The mean ± standard deviation was used for continuous variables and percentage for categorical variables as according to distribution state. Mann–Whitney U-test or Student's *t*-test was used to compare two independent groups according to distribution state. Spearman's correlation coefficients were used to evaluate statistical significance among nonnormally distributed variables (TRX and TXNIP).

## Results

### Baseline characteristics of the study subjects

The mean age of 45 patients and 33 healthy controls was 40 ± 16 and 41 ± 14 years, respectively. No significant difference was observed between the two groups with respect to age. Table 1 summarizes the demographic and laboratory characteristics of the patients with AIS.

Our results indicated that serum TXNIP levels were significant higher in patients with AIS (3.98 ± 0.19 ng/mL [mean ± standard error mean (SEM)], $n = 45$) than in healthy controls (3.3 ± 0.23 ng/mL [mean ± SEM], $n = 33$; $P = 0.044$ Figure 1a). However, no significance difference was observed in serum TRX levels between stroke patients (11.9 ± 0.63 ng/mL [mean ± SEM], $n = 45$; $P = 0.405$) and healthy controls (11.0 ± 0.73 ng/mL [mean ± SEM], $n = 33$ Figure 1b). Moreover, a positive correlation was observed between TXNIP and TRX levels [$R^2 = 0.476$, $P < 0.003$; Figure 2].

## Discussion

The main findings of the present study are as follows: (1) TXNIP levels are significantly higher in stroke patients than in healthy controls and (2) TRX levels are not significantly different between stroke patients and healthy controls.

Oxidative stress is associated with the pathogenesis of many diseases, including cancer, neurodegenerative disease, aging, and stroke.[5] Production of free radicals (ROS) contributes to brain damage in stroke and reperfusion stroke.[4] The brain is more prone to oxidative stress than other organs because of the presence of polyunsaturated fatty acids; high consumption of oxygen by brain cells; and decreased activities of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione reductase, thus making the brain an easy target for attack by ROS.[15] Accumulating evidence suggests that oxidative stress plays an important role in stroke and reperfusion stroke.[4,16,17] Toxic effects of the oxidative system are neutralized by the action of the enzymatic and nonenzymatic antioxidant system.[3] The TRX/TXNIP system in the central nervous system regulates many biological

**Table 1: Comparison of demographic parameters**

| Variables | Patients | Healthy controls | *P* |
|---|---|---|---|
| Age (years) | 40±16 | 41±11 | 0.125 |
| Oral temperature (°C) | 36.9±0.2 | 36±0.1 | 0.623 |
| Peripheral pulse rate | 83±13.7 | 82±12 | 0.121 |
| Weight (kg) | 77±19.6 | 84±14.4 | 0.072 |
| Height (cm) | 165±9.5 | 170±6 | 0.093 |
| BMI (kg/m$^2$) | 27.8±6.2 | 28.7±3.9 | 0.112 |

BMI: Body mass index

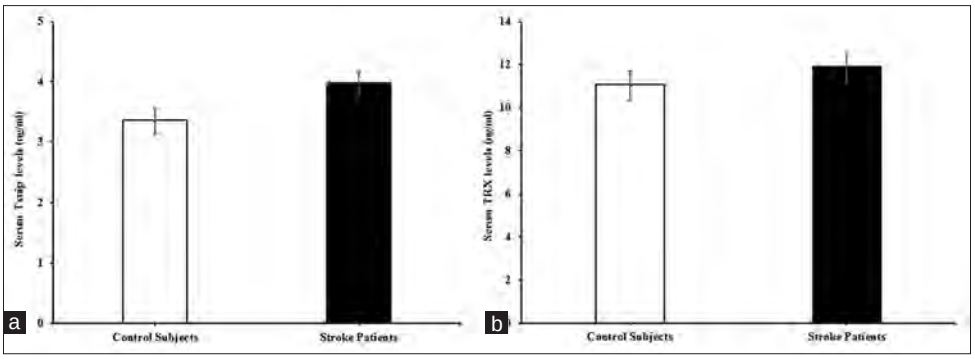


**Figure 1:** (a) Analysis of serum levels of thioredoxin-interacting protein levels in acute ischemic stroke patients and healthy controls by indirect ELISA. Serum thioredoxin-interacting protein levels were significantly higher in stroke patients than in healthy controls ($P = 0.044$). (b) Analysis of serum thioredoxin levels between acute ischemic stroke patients and healthy controls by indirect ELISA. There was no significant difference in thioredoxin serum levels of stroke and healthy participants ($P = 0.405$)

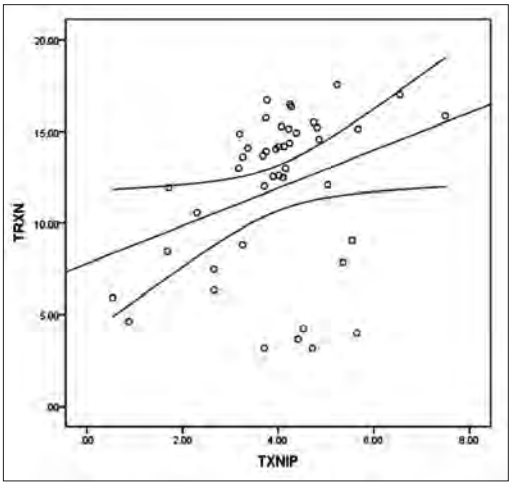


**Figure 2:** Correlation of thioredoxin-interacting protein and thioredoxin levels in stroke patients. Comparison between thioredoxin-interacting protein and thioredoxin showed positive correlation ($R^2 = 0.476$)

processes and signaling pathways in the brain.[18] TRX is a redox protein that exerts antioxidant effects through TRX reductase and NADPH, a reducing agent. Effects of TRX are regulated by TXNIP, which in turn regulates the cellular redox state.[19]

In the present study, no significant difference was observed in TRX levels between stroke patients and healthy controls. A recent study reported that TRX levels were elevated in Chinese patients with AIS and were associated with stroke severity and lesion volume.[20] This study suggested that TRX can be considered as a novel independent diagnostic marker for AIS. Our results are not in agreement with the above study. Possible reasons for the discrepancy in study results is the small sample size included in our study compared with that included in the study by Wu *et al*. (312 patients)[20] and difference in the population cohort between these two studies. Therefore, further studies involving a large sample size should be performed to replicate the findings of the present study.

In the present study, TXNIP levels were statistically higher in stroke patients than in healthy controls. TXNIP is an endogenous inhibitor and regulator of the TRX system. It is possible that stroke patients in the severe phase of the disease develop high oxidative stress. Significantly elevated TXNIP expression is associated with proinflammatory and proapoptotic responses in stress-related disease models of neurotoxicity, metabolic disorder, and stroke.[10,21] An earlier study had shown elevated levels of tumor necrosis factor-alpha mRNA and protein in ischemic brain.[22] It is believed that during ischemic stroke, NOD-like receptor protein 3 inflammasome in neurons and glial cells has a role in detecting tissue damage and induces inflammatory response. A recent animal study showed that TXNIP contributed to the development of acute ischemic brain injury by inducing redox imbalance and inflammasome activation and that therapeutic inhibition of TXNIP prevented ischemic brain injury by reducing inflammatory processes.[14] Thus, TXNIP induces neurotoxicity by releasing proinflammatory cytokines and altering antioxidant status.[10,23]

The present study is the first to report significantly increased TXNIP levels in AIS patients and to suggest that TXNIP levels can be used as a useful biomarker in this region. However, it is unclear whether significantly high TXNIP levels in AIS patients have any pathological significance, i.e., whether they are a risk factor of AIS onset or simply an indicator of oxidative stress and inflammation. Therefore, further large-scale studies involving increased number of patients are required to investigate the role of TXNIP in AIS.

## Limitations/recommendations

1. TRX and TXNIP levels were measured in a small population. Therefore, further studies involving a large sample size should be conducted to validate the results of the present study
2. TRX and TXNIP levels were determined at a single time point. These findings did not reflect when and how the levels of these markers change and it is possible that our data might underestimate the fluctuations in the levels

of oxidative stress. Therefore, serial measurements of circulating TRX and TXNIP levels should be performed under pre- and post-AIS conditions

3. TRX and TXNIP levels were measured using the serum and not the cerebrospinal fluid. Therefore, it is unclear whether these levels reflect similar changes in the central nervous system.

## Conclusions

Novel biomarkers are needed to determine the level of disease in stroke patients. To the best of our knowledge, this is the first clinical study to report increased TXNIP levels in acute ischemic stroke patients in local population.

### Financial support and sponsorship

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding this work through research group No (RG- 1438-008).

### Conflicts of interest

There are no conflicts of interest.

## References

1. Feigin VL, Norrving B, George MG, Foltz JL, Roth GA, Mensah GA, *et al.* Prevention of stroke: A strategic global imperative. Nat Rev Neurol 2016;12:501-12.
2. Alahmari K, Paul SS. Prevalence of stroke in Kingdom of Saudi Arabia – Through a physiotherapist diary. Mediterr J Soc Sci 2016;7:228-33.
3. Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative stress and antioxidant defense. World Allergy Organ J 2012;5:9-19.
4. Allen CL, Bayraktutan U. Oxidative stress and its role in the pathogenesis of ischaemic stroke. Int J Stroke 2009;4:461-70.
5. Chan PH. Role of oxidants in ischemic brain damage. Stroke 1996;27:1124-9.
6. Nakamura H. Thioredoxin and its related molecules: Update 2005. Antioxid Redox Signal 2005;7:823-8.
7. Taniguchi Y, Taniguchi-Ueda Y, Mori K, Yodoi J. A novel promoter sequence is involved in the oxidative stress-induced expression of the adult T-cell leukemia-derived factor (ADF)/human thioredoxin (Trx) gene. Nucleic Acids Res 1996;24:2746-52.
8. Xie Z, Sun J, Li H, Shao T, Wang D, Zheng Q, *et al.* Plasma and synovial fluid trxR levels are correlated with disease risk and severity in patients with rheumatoid arthritis. Medicine (Baltimore) 2016;95:e2543.
9. Burke-Gaffney A, Callister ME, Nakamura H. Thioredoxin: Friend or foe in human disease? Trends Pharmacol Sci 2005;26:398-404.
10. Al-Gayyar MM, Abdelsaid MA, Matragoon S, Pillai BA, El-Remessy AB. Thioredoxin interacting protein is a novel mediator of retinal inflammation and neurotoxicity. Br J Pharmacol 2011;164:170-80.
11. Griffiths HR, Bennett SJ, Olofsson P, Dunston CR. Thioredoxin as a putative biomarker and candidate target in age-related immune decline. Biochem Soc Trans 2014;42:922-7.
12. Nakamura H, De Rosa S, Roederer M, Anderson MT, Dubs JG, Yodoi J, *et al.* Elevation of plasma thioredoxin levels in HIV-infected individuals. Int Immunol 1996;8:603-11.
13. Drake C, Boutin H, Jones MS, Denes A, McColl BW, Selvarajah JR, *et al.* Brain inflammation is induced by co-morbidities and risk factors for stroke. Brain Behav Immun 2011;25:1113-22.
14. Ishrat T, Mohamed IN, Pillai B, Soliman S, Fouda AY, Ergul A, *et al.* Thioredoxin-interacting protein: A novel target for neuroprotection in experimental thromboembolic stroke in mice. Mol Neurobiol 2015;51:766-78.
15. Aon-Bertolino ML, Romero JI, Galeano P, Holubiec M, Badorrey MS, Saraceno GE, *et al.* Thioredoxin and glutaredoxin system proteins-immunolocalization in the rat central nervous system. Biochim Biophys Acta 2011;1810:93-110.
16. Saeed SA, Shad KF, Saleem T, Javed F, Khan MU. Some new prospects in the understanding of the molecular basis of the pathogenesis of stroke. Exp Brain Res 2007;182:1-0.
17. Tsai NW, Chang YT, Huang CR, Lin YJ, Lin WC, Cheng BC, *et al.* Association between oxidative stress and outcome in different subtypes of acute ischemic stroke. Biomed Res Int 2014;2014:256879.
18. Patenaude A, Murthy MR, Mirault ME. Emerging roles of thioredoxin cycle enzymes in the central nervous system. Cell Mol Life Sci 2005;62:1063-80.
19. Mohamed IN, Hafez SS, Fairaq A, Ergul A, Imig JD, El-Remessy AB, *et al.* Thioredoxin-interacting protein is required for endothelial NLRP3 inflammasome activation and cell death in a rat model of high-fat diet. Diabetologia 2014;57:413-23.
20. Wu MH, Song FY, Wei LP, Meng ZY, Zhang ZQ, Qi QD, *et al.* Serum levels of thioredoxin are associated with stroke risk, severity, and lesion volumes. Mol Neurobiol 2016;53:677-85.
21. Kim GS, Jung JE, Narasimhan P, Sakata H, Chan PH. Induction of thioredoxin-interacting protein is mediated by oxidative stress, calcium, and glucose after brain injury in mice. Neurobiol Dis 2012;46:440-9.
22. Liu T, Clark RK, McDonnell PC, Young PR, White RF, Barone FC, *et al.* Tumor necrosis factor-alpha expression in ischemic neurons. Stroke 1994;25:1481-8.
23. Devi TS, Lee I, Hüttemann M, Kumar A, Nantwi KD, Singh LP, *et al.* TXNIP links innate host defense mechanisms to oxidative stress and inflammation in retinal muller glia under chronic hyperglycemia: Implications for diabetic retinopathy. Exp Diabetes Res 2012;2012:438238.

Original Article

# Organ Donation Awareness and Attitude among Riyadh City Residents, Saudi Arabia

**Aws Almufleh, Rasha Althebaity[1], Ali S. Alamri[2], Nada A. Al-Rashed[3], Eman H Alshehri[4], Lina Albalawi[5], Reem Alameer[6], Eman Hajr[7], Ismail A. Raslan[8], Faisal A Alsaif[9]**

Cardiac Sciences Department, King Fahad Cardiac Center, King Saud University, Riyadh, [1]Department of Surgery, College of Medicine, Majmaah University, Al Majmaah, [2]Department of Surgery, King Saud Medical City, [3]General Surgery Registrar in Security Forces Hospital, [4]Department of Obstetrics and Gynecology, College of Medicine, King Saud University, Riyadh, [5]Demonstrator, Internal Medicine Department, Almajmaah University, Al Majmaah, [6]Infectious Diseases Section, Internal Medicine Department, King Faisal Specialist Hospital and Research Center, [7]Saudi Board in Otorhinolaryngology Head and Neck Surgery, Otology/Neurotology Fellow, Al Imam Mohammed Bin Saud University, [8]Department of Internal Medicine, College of Medicine, [9]Department of Surgery, College of Medicine, King Saud University, Riyadh, Saudi Arabia

## Abstract

**Introduction:** Organ donation is widely contentious among the Saudi population though remains largely understudied. In the aim to understand the public perception of organ donation, willingness to donate, and the reasons for donation refusal, we carried out this study. **Methodology:** A cross-sectional questionnaire-based study was conducted on a stratified-by-region random sample of 2596 Saudi residents in Riyadh area between 15 and 70 years of age in March 2010. The main outcomes were rates of organ donation awareness, willingness to donate, and awareness of Islamic opinion permitting organ donation. Secondary exploratory analysis was performed to determine reasons for organ donation refusal. **Results:** Seventy-six percentage of the sample had some background knowledge of organ donation; however, 41% were unwilling to donate their organs, with only 30.1% of our sample having had a prior knowledge about Islamic opinion about organ donation. Overall, of those who had background knowledge of organ donation, 79.5% thought that organ donation was important or very important. Respondents who are women, older, more educated, and in higher income group were more likely to be aware of organ donation ($P < 0.05$) and those younger than 35 years old were more likely to be unaware of the Islamic opinion ($P < 0.001$). The most cited reasons for donation refusal included the desire to be buried with complete parts (43.8%) (i.e., not disfigured), having an incomplete idea about brain death (24%), and because they thought that it was forbidden in Islam (15.1%). **Conclusion:** The level of organ donation awareness was comparatively high, but knowledge of the Islamic views of organ donation lacked among high portion of our sample, which partially explains the high organ donation refusal rate. There remains a large need to promote public awareness about the importance of organ donation and to clear the confusion of the Islamic view.

**Keywords:** Donation awareness, organ donation, organ transplantation

## Introduction

Organ transplantation is currently recognized to be the treatment of choice for multiple end-stage organ diseases.[1] The need for organ transplantations is rapidly increasing in Saudi Arabia. For instance, the number of end-stage renal disease patients in Saudi Arabia approached 12,116 in 2011 and is predicted to double in the next decade.[2,3] The expected posttransplantation 5-year survival rates are 85% for kidney transplant, 70% for liver transplant, and 65% for heart transplant,[4] which are much higher when compared to the conventional supportive therapy. According to the Saudi Center for Organ Transplantation latest statistics, 309 of patients undergoing brain death in Saudi Arabia were eligible for organ donation, but unfortunately only 23% of those patients' families agreed to donate their organs. An even lesser rate was reported in a study reviewing 162 brain-dead patients' families' response when approached for organ donation in King Abdulaziz Medical City, where only 17%

**Access this article online**

Quick Response Code:

**Website:** www.jnsmonline.org

**DOI:** 10.4103/JNSM.JNSM_5_18

**Address for correspondence:** Dr. Faisal Abdullah Alsaif, HPB and Transplant Surgery, College of Medicine, King Saud University, Riyadh, Saudi Arabia.
E-mail: falsaif@ksu.edu.sa



**How to cite this article:** Almufleh A, Althebaity R, Alamri AS, Al-Rashed NA, Alshehri EH, Albalawi L, *et al*. Organ donation awareness and attitude among Riyadh City Residents, Saudi Arabia. J Nat Sci Med 2018;1:59-63.

agreed for donation.[1] The reason for family refusal differs widely, but a common denominator was found to be the lack of understanding of the concept of brain death[2] and misconception of the Islamic view on organ donation.

A study carried out in Riyadh and Jeddah cities in Saudi Arabia in 1995 revealed that 68% of respondents were willing to donate their relatives' organs in case they endured brain death and 38% agreed to donate their own organs.[5,6] Not unexpectedly, a recently published systematic review has shown religious opinion to be a very important factor to affect organ donation in the Middle East.[6,7] The above studies were mostly from single-center and covered small nonrepresentative samples, which highlights the need for larger study to establish a better understanding of rates of organ donation awareness, willingness to donate, and awareness of Islamic opinion.

## Methodology

The study was approved by King Saud University Ethics Review Board; it was an observational cross-sectional study that targeted a stratified-by-region random sample taken from the population of Riyadh city and surrounding villages, i.e., Riyadh area. Riyadh city was geographically sectioned into four quarters, i.e., south, west, east, and north. Within each quarter, based on local residents' advice about the most populated malls, schools and colleges were targeted. Outside the city, concentric villages were chosen to complement the urban Riyadh city with its rural surroundings. The study was conducted in a 2-week period in March 2010 in association with educational campaign aimed to increase the awareness of Saudi society about organ donation. The activities of campaign took place in several sites, for example, schools, universities, colleges, and main malls. Age range was between 15 and 70 years. The target population approached was 3000 male and female participants, 2596 out of which completed our questionnaire. The remaining 404 participants were excluded as they either did not return their questionnaires or had incompletely filled them. We utilized list-wise deletion to handle questionnaires with missing data.

A standardized questionnaire was used to ascertain the awareness, knowledge, and attitude toward organ donation and transplantation as well as few background and demographic questions. The questionnaire was based on several previous validated questionnaires used in previous studies.[5,8,9] It was then tested on a pilot of 65 individuals who were chosen randomly from King Saud University Center. The format and content were found to be clear and straightforward, suggesting no further edition on the questionnaires. They were distributed by trained personnel who had a clear idea about research questions, choices, and possible answers. It was ascertained that questionnaires were filled by participants before attending the activity of educational program and without influence on their opinions by the members of campaign.

Data were entered and analyzed using the SPSS Inc. Released 2008. SPSS Statistics for Windows, Version 17.0. (Chicago, US: SPSS Inc). Chi-square tests of independence were performed to test the association between the various study variables. Based on the Chi-square test results, odds ratios (ORs) were calculated and reported along with the 95% confidence interval. $P < 0.05$ was considered statistically significant.

## Results

The study targeted 3000 recipients, 2596 of whom returned the questionnaires filled, making the response rate of 86.5%.

As shown in Table 1, while the majority of our population were from Riyadh area, were students, and were 15–20 years of age, our sample spectrum included substantial numbers from all categories across the Saudi demographics, students, workers, and unemployed people of both genders, and all socioeconomic and occupational levels were included.

When asked about whether they heard about organ donation or not before, 76.3% said yes, while only 23.7% never heard about organ donation prior to the campaign. Awareness level was found to be higher among older participants, with a statistically significant difference ($P = 0.01$). Table 2 shows the participants' answer to most of the study questions.

The degree of perceived importance of organ donation was important or very important in 79.8%, but neutral in 16.9% and unimportant in 3.5%.

In response to the question regarding willingness to agree to organ donation of a relative if they had brain death, 56% agreed, 16% did not, while 27.4% remained undecided. The reason behind the willingness to donate organs was humanity

**Table 1: Demographical data of the study participants**

| Demographic variable | Distribution | Frequency (%) |
|---|---|---|
| Age (years) | 15-20 | 1337 (51.5) |
| | 21-35 | 973 (37.5) |
| | 36-70 | 285 (11) |
| Gender | Male | 1218 (46.9) |
| | Female | 1377 (53.0) |
| Residence | Riyadh | 2407 (92.8) |
| | Outside | 188 (7.2) |
| Marital status | Married | 633 (24.4) |
| | Single | 1905 (73.3) |
| | Divorced | 41 (1.5) |
| | Widowed | 16 (0.6) |
| Educational level | Uneducated | 33 (1.3) |
| | School | 921 (35.6) |
| | Higher education | 1641 (63.2) |
| Occupation | Unemployed | 340 (13) |
| | Student | 1885 (72.6) |
| | Employed | 370 (14.2) |
| Family income | <5000 SR | 190 (7.3) |
| | 5000-10,000 SR | 720 (27.7) |
| | 10,000-15,000 SR | 721 (27.8) |
| | >15,000 SR | 964 (37.1) |

SR: Saudi riyal

and giving reasons in 47%, religious rewards in 37%, and because they witnessed someone in need to organs 13.7%. On the other hand, among those who refused donation, 27.7% reported the fear to be buried "incomplete," 17.7% thought the donor's body will be disfigured, 19.7% reported the disagreement of family in this issue, 19.4% refused because they lacked the basic understanding of brainstem death, and finally 15% refused donation because they thought it was forbidden in Islam.

About 70% of our study participants were unaware of the presence of a religious opinion permitting organ donation, while only 30% had prior knowledge about it. Rate of lack of awareness of Islamic opinion was higher in younger participants (<20 years of age), $P < 0.001$, OR =1.603).

## Discussion

Our study is, by far, the largest in Saudi Arabia investigating organ donation awareness in the general population. The level of organ donation awareness in our study (74%) was higher than the previous national study which estimated awareness to be 44% among urban and 31% among rural populations. A possible explanation to the higher level of awareness in our study is the increasing number of organ donation awareness campaigns which is proposed to be a key factor in improving awareness.[10] Our study, however, in contrast to previous national studies, reported no statistically significant difference [Table 3] between Riyadh and its surrounding rural villages, which probably reflects the fast spread of knowledge to Riyadh's neighboring villages and the higher rate of villages' urbanization.

Reported willingness to donate brain-dead relatives' organs was 56% in our study, about 12% lower than the previous 1996 national-based study, a discrepancy that can be explained by the limited awareness of religious opinion in our sample (30% only) compared to 42% in the 1996 study.[5] Reasons for refusal that included body disfigurement, religious opinion

**Table 2: Participants' answer to main study questions**

| Research question | Yes (%) | No (%) | Don't know (%) |
|---|---|---|---|
| Have you heard about organ donation? | 1980 (76.3) | 615 (23.7) | - |
| Are you aware about brain death concept? | 2331 (89.8) | 265 (10.2) | - |
| Would you agree to donate a relative's organs if he/she endured brain death? | 144 (55.5) | 428 (16.5) | 726 (27.9) |
| Are you aware about the Islamic opinion regarding organ donation? | 776 (29.9) | 831 (32.0) | 989 (38.1) |
| Would you agree to donate your own organs during life? | 576 (22.18) | 1360 (52.4) | 658 (25.35) |
| Would you agree to donate your organs if you endured brain death? | 1310 (50.46) | 628 (24.19) | 658 (25.35) |

**Table 3: Factors affecting level of organ donation awareness across our population and the statistical significance of their interaction**

| Factor affecting awareness | Awareness levels | | *P* | OR |
|---|---|---|---|---|
| | Aware (%) | Not aware (%) | | |
| Gender | | | | |
| Male | 1102 (90.4) | 117 (9.6) | <0.0001 | 0.935 (0.91-0.954)* |
| Female | 1332 (96.7) | 45 (3.3) | | 1.97 (1.53-2.533)* |
| Place of residence | | | | |
| Riyadh | 2264 (94) | 144 (6) | 0.051 | |
| Surrounding villages | 170 (90.4) | 18 (9.6) | | |
| Marital status | | | | |
| Married | 610 (95.9) | 26 (4.1) | 0.010 | 1.56 (1.09-2.236)* |
| Single | 1824 (94) | 136 (7) | | 0.589 (0.391-0.88)* |
| Family income | | | | |
| <5000 SR/month | 160 (84) | 30 (16) | <0.0001 | 0.267 (0.164-0.436)* |
| 5000-10,000 SR/month | 671 (93) | 49 (7) | | 0.686 (0.453-1.039)* |
| 10,000-15,000 SR/month | 685 (94.5) | 37 (5.5) | | 0.928 (0.595-1.446)* |
| >15,000 SR/month | 918 (95) | 46 (5) | | 1.00 (reference) |
| Level of education | | | | |
| Illiterate | 28 (82.4) | 6 (17.6) | <0.0001 | 0.271 (0.77-0.95)* |
| Primary school | 24 (85.7) | 4 (14.3) | | 0.349 (0.087-1.40)* |
| Junior high school | 98 (91.6) | 9 (8.4) | | 0.633 (0.204-1.96)* |
| High school | 718 (91.5) | 67 (8.5) | | 0.623 (0.244-1.58)* |
| Bachelor degree | 1480 (95.4) | 71 (4.6) | | 1.212 (0.477-3.08)* |
| Master degree and above | 86 (94.5) | 5 (5.5) | | 1.00 (reference) |

*95% CI. CI: Confidence interval, OR: Odds ratio, SR: Saudi riyal

**Table 4: Comparison between levels of awareness and willingness for organ donation across five large countries**

| Research question | Present study | Greece | US | India | Poland | China |
|---|---|---|---|---|---|---|
| Sample size, men (%) | 2596 (46.9) | 224 (46.5) | 2056 (49) | 123 (54) | 1100 (50) | 2930 (42) |
| Have you heard about organ donation? (level of organ donation awareness) | 76.3% | 52.9% | 94% | 96.5% | 82% | 95% |
| Would you agree to donate a relative's organs if he/she endured brain death? | 55.5% | NA* | 52.8% | 69% | NA* | 90% |
| Would you agree to donate your own organs during life? | 22.18% | 71% | NA* | NA* | 50% | 65% |
| Would you agree to donate your organs if you endured brain death? | 50.46% | 45.7% | 80% | 89% | NA* | 73% |

*NA, questions not mentioned in those studies

unawareness, and family disagreement were consistent with the two previous studies.[5,8]

Organ donation awareness was found to be significantly more in women, higher educated individuals, those with higher socioeconomic status, and in married people [Table 3]. This goes in synchronization with many previous studies[11-13] that identified those to be strong predictors for higher levels of organ donation awareness.

All studies of organ donation awareness in Saudi Arabia, including ours, have consistently yielded lower awareness (30%–74%) levels than most of its counterparts around the world [Table 4].[14-17] However, compared to previous studies in Saudi Arabia, the increased rate of organ donation awareness in our study reflects a promising trend and a higher chance of having next-of-kin agreement to organ donation in case of a relative's brain death. As the morbidity of end-organ dysfunction continues to plague the country, improvement of organ donation awareness in our study, albeit marginal, represents a step in the right direction. However, the knowledge of Islamic opinion was only 30% which calls for more awareness campaigns that include Islamic education about the permissibility of organ donation.

Finally, although our study has a considerably large sample size and included both urban and rural areas, it is important to note some limitations. First, our study design was observational with its inherent limitations. Second, the study only included the central region of Saudi Arabia; therefore, caution should be exercised before generalizing its findings to the rest of the country. Furthermore, 13.5% of the participants approached did not return completed questionnaires and therefore were excluded; whether this impacts the true rate of organ donation awareness is unknown.

## Conclusion

Disseminating the idea of the organ donation, explaining the concept of brain death, and utilizing mass media in that purpose are a cornerstone in improving the culture of organ donation in the long term. Larger, more comprehensive study covering all Saudi Arabian regions is needed to help us understand the full dimension of this issue in the country.

### Acknowledgment

The research team would like to acknowledge the contributions by Ahmed M. Alaqeel, Omar M. BenHusain, Rawabi H. Alhathlool, Adel A Alahaidib, Bilal Marwa, Mohammed Alotaibe, Saad A Bin Ayeed, Faiza Alotaibi, Afnan Almass, Amjad Aldrees, Dana Aldehmashy, Sarah Hawidi, Asma Alaqeel, Auroabah S. AlMufleh, and Raed Faraj. We are grateful to Dr. Salwa Tayel for her help in coding system of the data and for her useful comments on an earlier version of the manuscript. We would also like to remark the amazing effort shown by "Save a Life" organ donation awareness campaign that followed our data collection and has probably had a major impact in improving organ donation and brain death concept awareness in our community.

### Financial support and sponsorship

Nil.

### Conflicts of interest

There are no conflicts of interest.

## References

1. Aldawood A, Al Qahtani S, Dabbagh O, Al-Sayyari AA. Organ donation after brain-death: experience over five-years in a tertiary hospital. Saudi journal of kidney diseases and transplantation : an official publication of the Saudi Center for Organ Transplantation, Saudi Arabia. 2007;18:60-4.
2. Shaheen FA, Souqiyyeh MZ. Current status of renal transplantation in the Kingdom of Saudi Arabia. Transplant Proc 2004;36:125-7.
3. Transplantation SCfO. Renal Replacement Therapy Organ Donation and Transplantation in the Kingdom of Saudi Arabia National Data 2011 SCOT annual Report. 2011;1:55-7.
4. Shaheen FA, Souqiyyeh MZ, Al-Attar B, Jaralla A, Al Swailem AR. Survey of opinion of secondary school students on organ donation. Saudi J Kidney Dis Transpl. 1996;7:131-4.
5. Shaheen FA, Souqiyyeh MZ. Improving transplantation programs and patient care. Transplant Proc 2005;37:2909-10.
6. Al Shehri S, Shaheen FA, Al-Khader AA. Organ donations from deceased persons in the Saudi Arabian population. Exp Clin Transplant 2005;3:301-5.
7. Newton JD. How does the general public view posthumous organ donation? A meta-synthesis of the qualitative literature. BMC Public Health 2011;11:791.
8. Alghanim SA. Knowledge and attitudes toward organ donation: a community-based study comparing rural and urban populations. Saudi J Kidney Dis Transpl 2010;21:23-30.
9. Bapat U, Kedlaya PG. Organ donation, awareness, attitudes and beliefs among post graduate medical students. Saudi J Kidney Dis Transpl 2010;21:174-80.
10. Rady MY, McGregor JL, Verheijde JL. Mass media campaigns and organ donation: managing conflicting messages and interests. Med Health Care Philos 2012;15:229-41.
11. Bilgel H, Sadikoglu G, Goktas O, Bilgel N. A survey of the public attitudes towards organ donation in a Turkish community and of the changes that have taken place in the last 12 years. Transpl Int 2004;17:126-30.
12. Manninen DL, Evans RW. Public attitudes and behavior regarding organ

donation. JAMA 1985;253:3111-5.
13. Ashraf O, Ali S, Ali SA, Ali H, Alam M, Ali A, *et al*. Attitude toward organ donation: a survey in Pakistan. Artif Organs. 2005;29:899-905.
14. Chen JX, Zhang TM, Lim FL, Wu HC, Lei TF, Yeong PK, *et al*. Current knowledge and attitudes about organ donation and transplantation among Chinese university students. Transplant Proc 2006;38:2761-5.
15. Wang W, Tian H, Yin H, Liu H, Zhang XD. Attitudes toward organ donation in China. Chin Med J (Engl) 2012;125:56-62.
16. Minniefield WJ, Muti P. Organ donation survey results of a Buffalo, New York, African-American community. J Natl Med Assoc 2002;94:979-86.
17. Symvoulakis EK, Komninos ID, Antonakis N, Morgan M, Alegakis A, Tsafantakis E, *et al*. Attitudes to kidney donation among primary care patients in rural Crete, Greece. BMC Public Health 2009;9:54.

Original Article

# Insomnia in Primary Care Settings: Still Overlooked and Undertreated?

**Aljohara S. Almeneessier[1,2], Bader N. Alamri[3], Faisal R. Alzahrani[4], Munir M. Sharif[2], Seithikurippu R. Pandi-Perumal[5], Ahmed S. BaHammam[2]**

Departments of [1]Family Medicine and [4]Otolaryngology Head and Neck Surgery, College of Medicine, King Saud University, [2]Department of Medicine, The University Sleep Disorders Center, College of Medicine, King Saud University, Riyadh, Saudi Arabia, [3]Department of Medicine, Dalhousie University, Halifax, [5]Somnogen Canada Inc, Toronto, Canada

## Abstract

**Background:** Insomnia is a major medical problem that is often associated with low health status and increased health-care utilization. Therefore, we conducted this study to determine the frequency of insomnia in a population presenting to the primary healthcare (PHC) clinics for health problems other than sleep disorders. **Methods:** We interviewed 336 consecutive patients attending PHC face-to-face by trained medical students. Validated questionnaires were used to evaluate insomnia, sleep quality, and daytime sleepiness. The insomnia questionnaire classifies patients into three categories: (1) no insomnia, (2) Level I insomnia with the absence of daytime dysfunction, and (3) Level II insomnia with the presence of daytime dysfunction. **Results:** Level I insomnia was reported by 19.3% and Level II by 57.1%. Patients with insomnia were older and had worse sleep quality. Apart from a higher prevalence of hypertension among patients with insomnia, there was no difference in other comorbidities between those with insomnia and no insomnia. None of the included patients has reported his/her complaint of insomnia to the treating general practitioner (GP), and none of them was diagnosed with insomnia by the GP. **Conclusion:** Insomnia and daytime dysfunction are very common in primary care population. Despite the frequent visits of these patients to PHC, none of them has reported that he/she complains to his/her GP, and therefore, did not receive any treatment for insomnia. Education of GPs is necessary to improve recognition, diagnosis, and treatment of insomnia.

**Keywords:** Daytime dysfunction, family physician, general practitioner, sleep, sleepiness

## Introduction

Insomnia is a common distressing sleep disorder. In fact, insomnia is the most common sleep disorder in the general population.[1] It is usually defined as difficulty in initiating or maintaining sleep, waking up earlier than desired, or nonrestorative sleep that results in significant distress or impairment in daytime function.[2] Some look to insomnia as a symptom of an underlying disorder rather than a disease on its own. Previously, chronic insomnia was viewed in some cases as secondary to underlying medical, psychiatric, neurological, or substance abuse disorders.[1] However, the recent approach to chronic insomnia best views the disorder as a comorbid disorder that warrants separate treatment attention.[3] Insomnia has been shown to be associated with serious medical complications. Several meta-analyses demonstrate that insomnia is a significant risk factor for cardiovascular diseases, arterial hypertension, myocardial infarction, chronic heart failure, and Type 2 diabetes.[3] Moreover, insomnia has been shown to be independently associated with significantly elevated use of health-care services, medications, and alcohol use.[4,5]

Several assessment tools have been used in epidemiological studies to assess the prevalence of insomnia, which results in a wide variance in the reported prevalence of the disorder.[6]

Primary care physicians are in a unique position to diagnose and to provide medical treatment for most patients with insomnia; nevertheless, insomnia, likewise most sleep disorders, is underrecognized and underdiagnosed.[7] Despite

**Address for correspondence:** Dr. Aljohara S. Almeneessier, Department of Family Medicine, College of Medicine, King Saud University, Riyadh, Saudi Arabia.
E-mail: aljoharas@yahoo.com

**Access this article online**

Quick Response Code:

**Website:** www.jnsmonline.org

**DOI:** 10.4103/JNSM.JNSM_30_18



**How to cite this article:** Almeneessier AS, Alamri BN, Alzahrani FR, Sharif MM, Pandi-Perumal SR, BaHammam AS. Insomnia in primary care settings: Still overlooked and undertreated?. J Nat Sci Med 2018;1:64-8.

that, insomnia is underrecognized, and many sufferers do not receive adequate treatment. Although insomnia is a distinct disorder, patients usually do not report insomnia symptoms to physicians, and physicians do not explore or assess sleep disorders in their patients.[8]

Therefore, we designed this study to determine the frequency of insomnia in a representative population presenting to the primary healthcare (PHC) clinics for health problems other than sleep disorders using a validated assessment questionnaire that has been validated in the primary care setting.

## Methods

### Sample

We conducted the study in six randomly selected (simple random) PHC centers in Riyadh city, Saudi Arabia in the period from October to December 2012. We targeted consecutive Saudi adult (>18 year) male and female patients attending the clinics with various medical problems. We excluded pregnant women and all shift workers, and workers forced to wake up early (before dawn (*Fajr*)) for professional duties. In addition, short sleepers, as defined in the 3rd edition of the International Classification of Sleep Disorders-3, were also excluded from the study.[2]

Medical records of the participants were checked to document the chronic illnesses of the participants and to verify whether the participants with insomnia have been diagnosed or treated for insomnia by their primary care physician.

Medical students explained the study protocol and objectives to the participants and interviewed the participants face-to-face. To minimize errors in data collection and diagnoses, medical students attended an educational session on insomnia and the used questionnaires, and received training on data collection.

Informed consent was obtained, and the institutional review board approved the study.

### Questionnaires

Demographic data and comorbidities were collected. In addition, sleep quality (Pittsburgh Sleep Quality Index [PSQI]) and daytime sleepiness (Epworth Sleepiness Scale [ESS]). The ESS is a validated questionnaire that assesses the likelihood that the subject will fall asleep during certain activities.[9] A score of ≥10 indicates daytime sleepiness. The PSQI is a validated and reliable questionnaire that is widely used as one of the most important sleep health assessment tools to assess sleep quality.[10] It is a self-report questionnaire that assesses sleep quality over a 1-month time interval. The measure consists of 19 individual items, creating seven components that produce one global score. The PSQI measures different aspects of sleep, including seven component scores and one composite score. The component scores consist of subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency (i.e., the percentage of time in bed that one is asleep), sleep disturbances, use of sleeping medication, and daytime dysfunction. Each item is scored on a 0–3 interval scale. The total PSQI score is then calculated by adding the seven component scores, to provide an overall score ranging from 0 to 21. A global score of ≥5 is associated with poor sleep. An Arabic version of the PSQI has been validated previously.[11]

To evaluate insomnia, a previously validated questionnaire to assess insomnia in the primary care setting was used.[12] The questionnaire is composed of five questions:

1. Do you often have difficulty falling asleep?
2. Do you wake up too early in the morning?
3. If you frequently wake up during the night, do you have difficulty going back to sleep?
4. Do you often feel tired when you awaken in the morning?
5. Does sleep loss affect your mood during the day, making you feel tense, irritable, or depressed?

Questions number 1, 2, and 3 aims to assess insomnia symptoms,[12] where participants were asked to report sleep difficulties persisting for at least 1 month.[2] For questions number 1, 3, 4, and 5, the frequency of symptom was considered on a weekly basis. Answers were considered positive if the complaint occurred three times or more per week. In question number 2, "too early in the morning" meant awakening before the patient's usual waking time, associated with an inability to return to sleep.

The clinical condition of the participants was classified according to the responses to the five questions. The clinical condition was classified as:

- No insomnia, if the answer was "no" to questions 1, 2, and 3
- Level 1 insomnia-an absence of daytime dysfunction, if the answer was "yes" to any of questions 1–3 and "no" to questions 4 and 5
- Level 2 insomnia-the presence of daytime dysfunction, if the answer was "yes" to any of questions 1–3 and "yes" to questions 4 or 5.

### Validation of the Arabic versions of the questionnaires

Two bilingual physicians independently translated the three used questionnaires from the original English versions into Arabic. Two other bilingual physicians, who had no knowledge of the original version of the questionnaire, back-translated the Arabic drafts into English. The translations were reviewed in collaboration with the translators, and the back-translation was assessed for equivalence to the original English versions. Discrepancies between the forward- and back-translation versions were discussed and resolved to produce the final Arabic versions. To maintain the psychometric properties of the original questionnaires, the questionnaires were at first administered to 10 bilingual subjects, who completed both the Arabic versions and the English versions to determine the test–retest reliability. Questionnaires results were independent of version. Afterward, we conducted a pilot study with a sample of 30 individuals to assess the legibility, practicality, and accuracy of the Arabic versions of the three questionnaires and the associated data gathering process before beginning the study.

### Statistical analysis

We chose a sample size that would allow us to detect an insomnia prevalence of 50% with an alpha ($\alpha$) of 0.05 and a precision of 1%. The estimated minimum sample size was 291.[12,13]

Data are expressed as means ± standard deviation; a Chi-square test was used to compare dichotomous data. For continuous variables, Student's *t*-test was used. We considered results significant if $P \leq 0.05$. We used SPSS 16.0 (Chicago, IL, USA) to analyze the data.

## Results

We recruited 336 participants, where males comprised 212 (63%), and the mean age of the participants was 33.2 ± 14.2 years with a mean body mass index of 26.9 ± 5.6. Criteria for Level I insomnia were met in 19.3% and Level II in 57.1%. Table 1 presents the demographics, educational levels, and social data of the total group, patients without insomnia, Level I insomnia and Level II insomnia.

Among patients with Level I insomnia, early morning awakening was reported in 78.5%, while difficulty in initiating sleep (36.9%) and maintaining sleep (29.2%) was less frequent. Among patients with Level II insomnia, difficulty falling asleep, difficulty in maintaining sleep, and early morning awakenings had high frequencies (58.3, 51.6, and 69.8%, respectively). Approximately 83% of patients with Level II insomnia exhibited a mood disturbance due to sleep loss, while 66.1% of patients with Level II insomnia complained of tiredness on awakening [Table 2].

Table 3 presents a comparison of comorbidities, the ESS and PSQI score of patients without insomnia, Level I insomnia and Level II insomnia. Patients with insomnia were older and had worse sleep quality. Apart from a higher prevalence of hypertension among insomniacs, there was no difference in other comorbidities between those with insomnia and no insomnia. Interestingly, participants had not been previously diagnosed or treated for insomnia, and none of them has reported his complaints of insomnia to the general practitioner.

**Table 1: Demographics, educational levels, and social data of the total group, patients without insomnia, Level I insomnia, and Level II insomnia**

| | Total (*n*=336) | Noninsomnia (*n*=79) | Level I insomnia (*n*=65) | Level II insomnia (*n*=192) |
|---|---|---|---|---|
| Age | 33.2±14.2 | 30.7±11.9 | 37.9±16.2 | 32.7±14 |
| BMI (kg/m$^2$) | 26.9±5.6 | 26.5±6.3 | 26.6±5.8 | 27.2±5.2 |
| Sex (males) (%) | 63 | 65 | 72 | 62.5 |
| Obese (BMI >30) (%) | 27.4 | 25 | 23 | 30 |
| Educational level (%) | | | | |
| No education | 3.9 | 1.3 | 6.2 | 4.2 |
| Elementary school | 5.4 | 5.1 | 7.7 | 4.7 |
| Intermediate | 10.1 | 6.3 | 10.8 | 11.5 |
| High school | 37.2 | 39.2 | 27.7 | 39.6 |
| University | 43.5 | 48.1 | 47.7 | 40.1 |
| Work | | | | |
| Employed | 42.6 | 31.6 | 55.4 | 42.7 |
| Retired | 6.8 | 3.8 | 10.8 | 6.8 |
| Nonemployed | 11.0 | 16.5 | 4.6 | 10.9 |
| Student | 28.6 | 32.9 | 21.5 | 29.2 |
| House wife | 8.3 | 10.1 | 6.2 | 8.3 |
| Other | 2.7 | 5.1 | 1.5 | 2.1 |
| Social status | | | | |
| Single | 46.4 | 50.6 | 35.4 | 48.4 |
| Married | 52.1 | 49.4 | 63.1 | 49.5 |
| Widowed | 0.6 | 0.0 | 0.0 | 1.0 |
| Divorced | 0.9 | 0.0 | 1.5 | 0.0 |
| Smoker history | | | | |
| X-smoker | 9.2 | 8.9 | 15.4 | 7.3 |
| Smoker | 13.4 | 13.9 | 20.0 | 10.9 |
| Daily Arabic coffee | 32 | 37 | 31 | 31 |
| Daily American coffee | 79 | 78 | 85 | 77 |
| Daily tea | 60 | 54 | 69 | 58 |

Except age and BMI, values are expressed in percentages. Level I insomnia, insomnia without day-time dysfunction. Level II insomnia, insomnia with daytime consequences. BMI: Body mass index

## Discussion

This is the first observational investigation on the distribution of insomnia in the primary care setting in Saudi Arabia. The study demonstrated that insomnia is extremely common in the primary care population and revealed a high prevalence of insomnia (76.4%), and most insomniacs (57.1%) complained of daytime disturbances as a result of their insomnia.

Our findings concur with an Italian study that used the same questionnaire and reported the presence of insomnia in 64% of primary care population, with 20% classified as Level I and 44% as Level II.[12]

The reported prevalence in both studies (the current study and the Italian study) is relatively high, which could be related to the fact that the used sleep questionnaire is based on a binary response (yes/no). This may have contributed to inflated estimates. In a previous review by Ohayon, the presence of symptoms of insomnia for at least three nights per week (similar to the definition of Level I insomnia in the current study) was reported in 16%–21% of the general population, while insomnia symptoms associated with daytime consequences (similar to a level II insomnia) was reported in 9%–15%.[13] However, in patients attending primary care with several comorbidities, the prevalence of insomnia is expected to be higher.

In a multisite survey of five American Medical Group Association, Level I and Level II insomnia were reported by 13.5 and 32.5% of the respondents, respectively.[14] In our study and the Italian

**Table 2: Insomnia symptoms**

| Insomnia symptoms questions[a] | Level I insomnia (*n*=65) | Level II insomnia (*n*=192) |
|---|---|---|
| Do you often have difficulty falling asleep? | 24 (36.9) | 112 (58.3) |
| If you frequently wake up during the night, do you have difficulty going back to sleep? | 19 (29.2) | 99 (51.6) |
| Do you wake up too early in the morning? | 51 (78.5) | 134 (69.8) |
| Do you often feel tired when you awaken in the morning? | 0 (0.0) | 127 (66.1) |
| Does sleep loss affect your mood during the day, making you feel tense, irritable, or depressed? | 0 (0.0) | 160 (83.3) |

[a]More than one answer per patient could be given. Level I: Insomnia, insomniacs without day-time dysfunction, Level II: Insomnia, insomniacs with daytime consequences

**Table 3: A comparison of comorbidities, the Epworth Sleepiness Score and the Pittsburgh Sleep Quality Index score of patients without insomnia, Level I insomnia and Level II insomnia**

| | Noninsomnia (*n*=79) | Level I insomnia (*n*=65) | Level II insomnia (*n*=192) | *P* |
|---|---|---|---|---|
| Age (year) | 30.7±11.9 | 37.9±16.2* | 32.7±14 | 0.007 |
| BMI | 26.5±6.3 | 26.6±5.8 | 27.2±5.2 | NS |
| Female gender (%) | 35.4 | 27.7 | 37.5 | NS |
| ESS | 6.6±4.1 | 5.1±3.9* | 6.8±4.4 | 0.02 |
| PSQI | 4.6±2.7 | 5.4±2.8* | 6.6±3.3* | <0.001 |
| Asthma (%) | 8.9 | 9.2 | 13.0 | NS |
| Hypertension (%) | 7.6 | 20.0 | 9.4 | 0.03 |
| Diabetes | 6.3 | 12.3 | 12.0 | NS |
| Anemia | 7.6 | 6.2 | 7.8 | NS |

**P*<0.05. BMI: Body mass index, ESS: Epworth Sleepiness Score, PSQI: Pittsburgh Sleep Quality Index, NS: Not significant

study,[12] where the survey was performed on patients attending PHC clinics, the percentage of patients who reported insomnia symptoms was high (76.4% and 64%, respectively), with a prevalence of Level II insomnia (57.1% and 44%, respectively) twice to three times that of Level I insomnia (20%). These results indicate that the effect of daytime dysfunction is an important factor that differentiates between subjects with insomnia in the general population and patients with insomnia in the primary care setting.

In developed countries, insomnia represents a heavy burden for primary care physicians. A previous study in the USA revealed that patients with insomnia ask for a clinical consultancy on average 12.87 times per year compared to 5.25 times per year for the patients without insomnia.[5] Previous studies in primary care settings in Saudi Arabia revealed that the majority of primary care physicians think that sleep disorders are related to psychiatric disorders.[7,15] However, psychiatric disorders are not the only risk factors for recurrent insomnia. In a survey carried out on a large sample of an adult population, multiple types of concomitant health problems were associated with increased difficulty in initiating and maintaining sleep.[16] Most patients attending primary care clinics have multiple health problems, which increase the risk for insomnia. Another major risk factor for the occurrence of insomnia was a previous complaint of insomnia.[16] Therefore, recurrent insomnia is not unusual in patients with treated insomnia. This requires proper follow-up and early treatment of recurrent insomnia in its early stages before becoming established and chronic, which stresses the importance of the role that primary care physicians can play in this perspective.

Although this study was conducted in a primary care setting, none of the interviewed participants with insomnia had been diagnosed or treated for insomnia; in addition, none of the patients has conveyed his complaint about poor sleep to the treating doctor suggesting that this disorder is underrecognized and undertreated. A previously published international survey examined the characteristics of insomnia in the general population in four developed countries (France, Italy, Japan, and the USA) to better understand why insomnia is underrecognized and undertreated.[17] The study showed that among those with a history of insomnia, the rate of reporting insomnia symptoms to their treating physicians was generally low, and among those who did consult a physician, few were prescribed any medication.[17] Not reporting symptoms of insomnia to the treating physicians needs further research. Different factors, such as culture and beliefs, may play a part in this. In addition, fear of the consequences and adverse effects of treatment including the possible risks of dependence on medications may be another factor. Underrecognition and undertreatment of insomnia in the primary care setting in this paper concur with a recent study in Saudi Arabia that revealed poor knowledge of sleep medicine and its disorders, and underrecognition of the importance and impact of sleep disorders among primary care physicians.[7] In addition, previous studies in the primary care settings in Saudi Arabia revealed that obstructive sleep apnea and restless legs syndrome are common among patients attending primary care services; however, these disorders are underrecognized by primary care physicians.[8,18,19] Therefore, educating primary care physicians on sleep disorders will allow the early detection of sleep disorders and the provision of proper treatment or referral to a sleep medicine specialist.

A limitation of this study is the fact that the study was conducted in a primary care setting, which limits our ability to generalize the results to a general Saudi population; however, this sample helped us to confirm the high prevalence of insomnia in the primary care settings and the underdiagnosis of this disorder by the treating physicians.

## Conclusion

This study reveals that insomnia and daytime dysfunction are very common in patients attending primary care clinics. Insomnia is a daily challenge for primary care physicians, who often miss and underdiagnose this disorder. Despite the high prevalence of insomnia among patients visiting primary care physicians, surveyed patients did not report their complaints to their treating physicians and therefore were unlikely to receive treatment or referrals to a specialty clinic. Focused and targeted education of primary care physicians on insomnia and other sleep disorders will allow the early detection of sleep disorders and hence the provision of early treatment and the prevention of complications.

### Financial support and sponsorship

This work was financially supported by the Strategic Technologies Program of the National Plan for Sciences and Technology and Innovation in the Kingdom of Saudi Arabia (MED511-02-08). The study sponsors played no role in the study design, the collection, analysis or interpretation of data, writing the manuscript, or the decision to submit the manuscript.

### Conflicts of interest

There are no conflicts of interest.

## References

1. Buysse DJ. Insomnia. JAMA 2013;309:706-16.
2. AASM. American Academy of Sleep Medicine (AASM). International classification of sleep disorders (ICSD), 3rd ed. Darien, IL: AASM. 2014.
3. Riemann D, Baglioni C, Bassetti C, Bjorvatn B, Dolenc Groselj L, Ellis JG, *et al*. European guideline for the diagnosis and treatment of insomnia. J Sleep Res 2017;26:675-700.
4. Bin YS, Marshall NS, Glozier N. The burden of insomnia on individual function and healthcare consumption in Australia. Aust N Z J Public Health 2012;36:462-8.
5. Sivertsen B, Krokstad S, Mykletun A, Overland S. Insomnia symptoms and use of health care services and medications: The HUNT-2 study. Behav Sleep Med 2009;7:210-22.
6. Luyster FS, Choi J, Yeh CH, Imes CC, Johansson AE, Chasens ER. Screening and evaluation tools for sleep disorders in older adults. Appl Nurs Res. 2015;28:334-40.
7. Saleem AH, Al Rashed FA, Alkharboush GA, Almazyed OM, Olaish AH, Almeneessier AS, *et al*. Primary care physicians' knowledge of sleep medicine and barriers to transfer of patients with sleep disorders. A cross-sectional study. Saudi Med J. 2017;38:553-9.
8. Bahammam AS, Al-Rajeh MS, Al-Ibrahim FS, Arafah MA, Sharif MM. Prevalence of symptoms and risk of sleep apnea in middle-aged Saudi women in primary care. Saudi Med J. 2009;30:1572-6.
9. Johns MW. A new method for measuring daytime sleepiness: The Epworth Sleepiness Scale. Sleep 1991;14:540-5.
10. Buysse DJ, Reynolds CF 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: A new instrument for psychiatric practice and research. Psychiatry Res 1989;28:193-213.
11. Suleiman KH, Yates BC, Berger AM, Pozehl B, Meza J. Translating the Pittsburgh Sleep Quality Index into Arabic. West J Nurs Res. 2010;32:250-68.
12. Terzano MG, Parrino L, Cirignotta F, Ferini-Strambi L, Gigli G, Rudelli G, *et al*. Studio Morfeo: insomnia in primary care, a survey conducted on the Italian population. Sleep medicine. 2004;5:67-75.
13. Ohayon MM. Epidemiology of insomnia: What we know and what we still need to learn. Sleep Med Rev 2002;6:97-111.
14. Hatoum HT, Kong SX, Kania CM, Wong JM, Mendelson WB. Insomnia, health-related quality of life and healthcare resource consumption. A study of managed-care organisation enrollees.
15. Bahammam AS. Knowledge and attitude of primary health care physicians towards sleep disorders. Neurosciences (Riyadh) 2001;6:59-62.
16. Klink ME, Quan SF, Kaltenborn WT, Lebowitz MD. Risk factors associated with complaints of insomnia in a general adult population. Influence of previous complaints of insomnia. Arch Intern Med 1992;152:1634-7.
17. Leger D, Poursain B. An international survey of insomnia: under-recognition and under-treatment of a polysymptomatic condition. Curr Med Res Opin. 2005;21:1785-92.
18. BaHammam A, Al-shahrani K, Al-zahrani S, Al-shammari A, Al-amri N, Sharif M, *et al*. The prevalence of restless legs syndrome in adult saudis attending primary health care. Gen Hosp Psychiatry 2011;33:102-6.
19. BaHammam AS, Alrajeh MS, Al-Jahdali HH, BinSaeed AA. Prevalence of symptoms and risk of sleep apnea in middle-aged Saudi males in primary care. Saudi Med J 2008;29:423-6.

Original Article

# The Impact of the "Brain Drain" Involving Saudi Physicians: A Cross-sectional Study

**Marya Alsuhaibani, Amjad Alharbi, Saleh K. Alqaryan[1], Turki Aldress[2], Majed Alharbi[3], Sami Alharethy[4]**

College of Medicine, Qassim University, Buraydah, [3]Department of Medicine, King Fahad Specialist Hospital, Qassim, [1]Department of Otolaryngology-Head and Neck Surgery, King Abdulaziz University Hospital, [4]Department of Otolaryngology-Head and Neck Surgery, College of Medicine, King Saud University, Riyadh, [2]Department of Otolaryngology-Head and Neck Surgery, College of Medicine, Prince Sattam Bin Abdulaziz University, Al-Saih, Saudi Arabia

## Abstract

**Objectives:** This study aimed to elucidate the brain drain phenomenon involving Saudi medical students by assessing their characteristics and intentions and related factors. **Materials and Methods:** A cross-sectional survey-based study conducted at Qassim University, Saudi Arabia. The subjects included 150 prefinal or final year medical students, who completed a modified version of a questionnaire developed by Akl *et al*. **Results:** Ninety-six students intended to study abroad with 48 and 33 planning to study in Canada and the USA, respectively. Country preference differed according to class ranking, and 69% and 33.3% of students in the top and bottom thirds of the class intended to study in Canada ($P = 0.047$). Male students were more likely to express the intention to study abroad. However, women were significantly more likely to remain abroad relative to males ($P < 0.001$). The only factor associated with intention to study abroad was the year of study and those in the final year were 60% less likely to express an intention to study abroad relative to those in prefinal years ($P = 0.012$). **Conclusion:** Most of our individuals intended to study abroad. It was varied according to gender differences. The primarily destination is Canada. This could present a challenge in meeting the high demand for staff in the health-care service in Saudi Arabia and exacerbate the current shortage of physicians in future.

**Keywords:** Brain drain, medical student, training abroad

## Introduction

Health-care providers are one of the cornerstones of health-care systems, and a sufficient supply of medical professionals is fundamental to the provision of healthcare.[1] The physician "brain drain" which involves physicians' emigration from developing countries with considerable shortages of health-care workers to developed countries has been a pressing issue and considered a potential threat to or crisis for health services in developing countries in recent years. The USA, Canada, the UK, and Australia are the top four destination countries for émigrés from developing or source countries and between 23% and 28% of physicians in these destination countries are international medical graduates.[2] African countries which are also source countries hold only 3% of the total number of health workers in the world but comprise 11% of the world's population.[3] On a domestic level, Lebanon has the highest proportion of medical graduates from a source country working outside their country.[4]

This issue not only affects the efficiency of health services but also leads to a loss of source countries' well-educated nationals who are required to lead the process involved in improving health systems to destination countries; in fact, 65% of all health professionals who emigrate are classified as "highly skilled."[5] The reasons underlying this issue are multiple and varied. For example, in a study conducted in Karachi, Syed *et al*. found that the quality of educational programs, remuneration, and poor work environments were the main reasons why physicians chose to work abroad.[6] In addition, a study conducted in Ireland showed that facilitation of specialty training pathways, improvements in pay and work environments and the provision of a clear career pathway for trainees could be implemented to address the problem.[1]

**Address for correspondence:** Dr. Marya Alsuhaibani, College of Medicine, Qassim University, Buraydah, Qassim, Saudi Arabia. E-mail: alsuhaibanima@gmail.com

**Access this article online**

Quick Response Code:

Website: www.jnsmonline.org

DOI: 10.4103/JNSM.JNSM_32_18



**How to cite this article:** Alsuhaibani M, Alharbi A, Alqaryan SK, Aldress T, Alharbi M, Alharethy S. The impact of the "Brain Drain" involving Saudi physicians: A cross-sectional study. J Nat Sci Med 2018;1:69-73.

Saudi Arabia as developing country is also subjected to this global issue. Saudi physicians working in Saudi Arabia percentage is accounted to be 21.5% ($n$ = 14,243) according to Ministry of Health Statistics. This low percentage of Saudi physicians may be affected by the brain drain phenomenon. Therefore, this study aimed to explore the kingdom's current position in terms of intervention policies and the magnitude of the problem.

## Materials and Methods

This cross-sectional survey-based study was conducted at Qassim College of Medicine in the Qassim region, Saudi Arabia. The individuals included 150 medical students in their prefinal and final years of college and voluntarily completed a modified version of a questionnaire developed by Akl *et al*.[4] The questionnaire was translated into Arabic to ensure the understanding of the students. Students were informed that the data would be used for research purposes only and the anonymity of the participants would be conserved. It is also indicated that filling the questionnaire would be considered as acceptance for participation in the study. The permission for distributing the survey was obtained from Qassim College of Medicine ethics committee. We distributed the questionnaire and obtained the results during November 2015.

### Statistical analysis

The mean value and standard deviation were calculated for individuals' age, and frequencies and proportions were calculated for all other variables. The relationships between student characteristics and three dependent variables representing students' intention to study abroad were explored. The dependent variables of interest included the intention to study abroad (yes or no), preferred destination country for intended study (Canada, the USA, or other), and whether students intended to return to Saudi Arabia (coded yes or no). However, as few respondents stated that they would remain abroad permanently, it was impossible to distinguish between those who intended to delay their return and those who planned never to return.

Univariate analyses including a *t*-test and an ANOVA were performed to compare age between groups. Other variables were compared using a Chi-square test or Fisher's exact test as appropriate. Intended specialties were recorded as "other" if they were chosen by fewer than 10 subjects to allow comparison. Multivariate analyses included logistic regression models involving backward elimination to identify characteristics associated with intention to study abroad and return to Saudi Arabia following training. In addition, a multinomial logistic regression model was used to analyze preferred destination countries for intended study. All tests were two-sided and $P < 0.05$ was considered statistically significant. The analyses were performed using Stata 11 MP.

## Results

The students' characteristics are reported in Table 1. Their mean age was 23.3 years and 101 were male students. Most students were single, and few considered themselves of lower socioeconomic status. In addition, most respondents ranked themselves in the middle third of their class in terms of academic performance.

**Table 1: Subject's baseline characteristics ($n$=150)**

| Variable | *n* (%) |
|---|---|
| Age, mean (SD) | 23.3 (1.2) |
| Sex | |
| Male | 101 (67.3) |
| Female | 49 (32.7) |
| Marital status | |
| Single | 142 (94.7) |
| Married | 8 (5.3) |
| Socioeconomic status | |
| Lower | 4 (2.7) |
| Lower middle | 32 (21.3) |
| Upper middle | 92 (61.3) |
| Upper | 22 (14.7) |
| Dual citizenship | |
| No | 131 (87.3) |
| Yes | 19 (12.7) |
| Year of medical school | |
| Prefinal | 79 (52.7) |
| Final | 71 (47.3) |
| Self-reported class rank | |
| Top third | 41 (27.3) |
| Middle third | 92 (61.3) |
| Bottom third | 17 (11.3) |
| Planned residency | |
| None (general practice) | 1 (0.7) |
| General surgery | 30 (20.0) |
| Internal medicine | 37 (24.7) |
| Dermatology | 4 (2.7) |
| Family medicine | 10 (6.7) |
| Psychiatry | 1 (0.1) |
| Pediatrics | 15 (10.0) |
| Obstetrics and gynecology | 1 (0.7) |
| Radiology | 10 (6.7) |
| Ear, nose, and throat | 1 (0.7) |
| Plastic surgery | 2 (1.3) |
| Ophthalmology | 6 (4.0) |
| Anesthesia | 1 (0.7) |
| Orthopedics | 10 (6.7) |
| Emergency medicine | 6 (4.0) |
| Other | 15 (10.0) |

SD: Standard deviation

Most respondents stated they intended to study abroad. Of the destination countries, Canada was chosen most frequently followed by the USA. In addition, most students stated that they would return to Saudi Arabia immediately following training [Table 2].

The relationship between preferred destination countries and intention to return are various according to countries. The proportion of students with a preference for Canada who stated that they would return home immediately following

**Table 2: Subject's postgraduate intentions (*n*=150)**

| Variable | *n* (%) |
|---|---|
| Intend to study abroad | |
| No | 54 (36.0) |
| Yes (specialty training) | 41 (27.3) |
| Yes (subspecialty training) | 55 (36.7) |
| Preferred destination country | |
| Canada | 48 (50.0) |
| France | 1 (1.0) |
| Germany | 7 (7.3) |
| UK | 7 (7.3) |
| USA | 33 (34.4) |
| Other | 1 (1.0) |
| Posttraining plan | |
| Return to Saudi Arabia immediately | 60 (62.5) |
| Work abroad for <5 years and return | 20 (20.8) |
| Work abroad for 5-10 years and return | 6 (6.3) |
| Work abroad for >10 years and return | 2 (2.1) |
| Never return | 8 (8.3) |

training was higher (77.1%, *n* = 37) relative to those observed in students who preferred the USA (45.6%, *n* = 15) or other countries (53.3%, *n* = 8), but this difference was not significant.

The intention to study abroad differed according to the year of study in medical school. The proportion of prefinal students who intended to study abroad was significantly higher relative to that of final year students [Table 3]. Destination country preference differed according to class ranking, and the proportion of students ranked in the top third of the class who intended to go to Canada was significantly higher relative to that of those ranked in the bottom third of the class. Students with dual citizenship were more likely to express the intention to study in the USA relative to those without dual citizenship. Moreover, male students were slightly more likely to express the intention to study abroad relative to women. However, women were significantly more likely to express the intention to remain abroad following training relative to male students.

The characteristics independently associated with the intention to study abroad in the multivariate analyses are reported in Table 4. As in the unadjusted analysis, the year of study in medical school was the only factor associated with the intention to study abroad and students in the final year were less likely to express the intention to study abroad relative to those in prefinal years. Male students and students with dual citizenship were more likely to express a preference for studying in the USA, rather than Canada, relative to female students and students without dual citizenship. In addition, class ranking was associated with country preference. Students who ranked lower in class were more likely to express a preference for studying in countries other than Canada relative to those who ranked higher in class. Moreover, the likelihood that male students would remain abroad following training was significantly lower relative to that observed for women.

## Discussion

The results showed that almost 70% of male students intended to study abroad but did not intend to remain abroad either for specialty or subspecialty training and most preferred to study in Canada. In contrast, approximately half of the women preferred to study in the USA and almost 70% intended to remain abroad following training. These findings are consistent with those of a study involving Lebanese students which showed that 40% of women intended to study abroad.[4] In addition, according to a study conducted by Akl *et al*. the proportion of female international medical graduates who were practicing physicians (30%) was lower relative to that of their male counterparts.[7] Moreover, the finding that the proportion of women who intended to study abroad was lower relative to that of male students could be attributed to cultural and social issues in Saudi Arabia which put some barriers in front of the woman such as having another person in the family to travel and stay with her abroad which sometimes is unavailable or difficult for that person due to his work.

The brain drain phenomenon is not exclusive to Saudi Arabia. A study conducted by Mullan showed that this type of migration occurred on a worldwide scale.[8] In the current study, approximately one-third of individuals did not intend to study abroad. This is consistent with the findings of Akl *et al*.'s study which showed that the majority (95.5%) of individuals intended to study abroad.[4] Moreover, a survey conducted in India included 166 final-year students showed that 59.0% of individuals had considered training abroad.[9] In one study, 4%, 2.1%, 1%, and 0.8% of medical graduates in Canada originated from the UK, India, Saudi Arabia, and Egypt, respectively.[10]

In addition, the current results showed that half and one-third of subjects intended to study in Canada and the USA, respectively. France was one of the less preferable destination countries in the current study. However, the proportion of Lebanese students in Akl *et al*.'s study who preferred to study in France (12.1%) was higher relative to that of those who preferred to study in Canada (4.2%).[4]

In the current study, 62.5% of students who intended to study abroad intended to return to Saudi Arabia immediately following training. However, women expressed a stronger intention to remain abroad following training relative to that observed in male students. In contrast, of the Lebanese students who intended to study abroad in Akl *et al*.'s study, 25.1% intended to return to Lebanon immediately following training and only 10.6% intended to remain abroad permanently.[4] From students' perspectives, the quality of the training provided was the primary factor in the decision to complete residency training and remain in the destination country permanently.

The results also showed that students' marital status affected the decision to study abroad. The proportion of single individuals who intended to study abroad (64.1%) was higher relative to that of married subjects (52.5%). The reason for this finding could be that single students have fewer

**Table 3: Relationships between student characteristics and intention to study abroad in univariate analyses**

| Variable | Intention to study abroad (*n*=96) | *P* | Intention to study in Canada (*n*=48) | Intention to study in the USA (*n*=33) | Intention to study in Europe (*n*=15) | *P* | Intention to remain abroad (*n*=36) | *P* |
|---|---|---|---|---|---|---|---|---|
| Age, mean (SD) | 23.3 (1.2)[a] | 0.627 | 23.2 (0.9) | 23.5 (1.5) | 22.9 (0.6) | 0.237 | 23.0 (1.3)[b] | 0.049 |
| Sex, *n* (%) | | | | | | | | |
| Male | 70 (69.3) | 0.052 | 39 (55.7) | 19 (27.1) | 12 (17.1) | 0.062 | 18 (25.7) | <0.001 |
| Female | 26 (53.1) | | 9 (34.6) | 14 (53.9) | 3 (11.5) | | 18 (69.2) | |
| Marital status, *n* (%) | | | | | | | | |
| Single | 91 (64.1) | 0.999 | 47 (51.7) | 29 (31.9) | 15 (16.5) | 0.139 | 34 (37.4) | 0.999 |
| Married | 5 (52.5) | | 1 (20.0) | 4 (80.0) | 0 | | 2 (40.0) | |
| Socioeconomic status, *n* (%) | | | | | | | | |
| Lower | 3 (75.0) | 0.774 | 1 (33.3) | 2 (67.7) | 0 | 0.971 | 0 | 0.454 |
| Lower middle | 21 (65.6) | | 10 (47.6) | 7 (33.3) | 4 (19.1) | | 8 (38.1) | |
| Upper middle | 60 (65.2) | | 31 (51.7) | 20 (33.3) | 9 (15.0) | | 22 (36.7) | |
| Upper | 12 (54.5) | | 6 (50.0) | 4 (33.3) | 2 (16.7) | | 6 (50.0) | |
| Dual citizenship, *n* (%) | | | | | | | | |
| No | 81 (61.8) | 0.202 | 37 (45.7) | 32 (39.5) | 12 (14.8) | 0.033 | 31 (28.3) | 0.717 |
| Yes | 15 (79.0) | | 11 (73.3) | 1 (6.7) | 3 (20.0) | | 5 (33.3) | |
| Year of medical school, *n* (%) | | | | | | | | |
| Prefinal | 57 (72.2) | 0.041 | 27 (47.4) | 23 (40.4) | 7 (12.3) | 0.265 | 25 (43.9) | 0.120 |
| Final | 39 (54.9) | | 21 (53.9) | 10 (25.6) | 8 (20.5) | | 11 (28.2) | |
| Self-reported class rank, *n* (%) | | | | | | | | |
| Top third | 29 (70.7) | 0.418 | 20 (69.0) | 8 (27.6) | 1 (3.5) | 0.047 | 11 (37.9) | 0.602 |
| Middle third | 58 (63.0) | | 25 (43.1) | 20 (34.5) | 13 (22.4) | | 23 (39.7) | |
| Bottom third | 9 (52.9) | | 3 (33.3) | 5 (55.6) | 1 (11.1) | | 2 (22.2) | |
| Planned residency, *n* (%) | | | | | | | | |
| General surgery | 22 (73.3) | 0.300 | 9 (40.9) | 6 (27.3) | 7 (31.8) | 0.260 | 12 (54.6) | 0.469 |
| Internal medicine | 26 (70.3) | | 13 (50.0) | 12 (46.2) | 1 (3.9) | | 9 (34.6) | |
| Family medicine | 0 | | | | | | | |
| Pediatrics | 10 (66.7) | | 7 (70.0) | 2 (20.0) | 1 (10.0) | | 2 (20.0) | |
| Radiology | 6 (60.0) | | 3 (50.0) | 3 (50.0) | 0 | | 2 (33.3) | |
| Orthopedics | 7 (70.0) | | 4 (57.1) | 1 (14.3) | 2 (28.6) | | 3 (42.9) | |
| Other | 25 (65.8) | | 12 (48.0) | 9 (36.0) | 4 (16.0) | | 8 (32.0) | |

[a]No intention: Mean=23.6 (1.3); [b]No intention: Mean=23.5 (1.1). SD: Standard deviation

responsibilities relative to those of married students and therefore find it easier to study abroad. In addition, the cost of studying abroad would be greater for married students relative to that incurred by single students. However, the number of married students included in this study is small in comparison to the single students which make the comparison between the two groups unfair.

Unexpectedly, socioeconomic status appeared to present less of a barrier to studying abroad for students of lower socioeconomic status. The proportion of these students (75%) who intended to study abroad was higher relative to that of students of upper and middle socioeconomic status 65.2%. The reason for this finding could be that many Saudi students benefit from scholarships offered by hospitals and universities in Saudi Arabia. In addition, students of lower socioeconomic status tend to go abroad to study and seek well-paying jobs on returning home.

The academic level has also an effect in the decision in going outside for training. In this study, the highest percentage of those intending to migrate was found in those with highest academic achievement. That is expected because the highest achievers will most likely be more equipped to perform better in the licensing examinations such as the United States Medical Licensing Examination and other examinations which are mandatory for acceptance in outside training; therefore, they have a higher chance of migrating.

The brain drain could have serious negative consequences for the quality of health-care services in Saudi Arabia. Moreover, it has implications for both policy and future research. This migration is associated with educational investment with uncertain return and a loss of intellectual capital. In fact, a number of high-income countries endeavor to address their own physician shortages by recruiting individuals from the pool of English-speaking international medical graduates

**Table 4: Multivariable analyses exploring the relationships between student characteristics and intentions to train abroad**

| Variable | Coefficient | *P* | OR | 95% CI |
|---|---|---|---|---|
| Intention to study abroad | | | | |
| Final year of medical school (reference=prefinal) | –0.39 | 0.012 | 0.40 | 0.20-0.82 |
| Destination country, USA (reference=Canada) | | | | |
| Dual citizenship (reference=none) | –2.28 | 0.043 | 0.10 | 0.01-0.93 |
| Male sex (reference=female) | –1.63 | 0.010 | 0.19 | 0.06-0.68 |
| Destination country, Europe (reference=Canada) | | | | |
| Class rank | | | | |
| Middle third (reference=top third) | 2.53 | 0.024 | 12.54 | 1.38-118.0 |
| Bottom third (reference=top third) | 2.53 | 0.133 | 12.57 | 0.46-341.7 |
| Intention to remain abroad | | | | |
| Male sex (reference=female) | –0.81 | <0.001 | 0.15 | 0.06-0.41 |

CI: Confidence interval, OR: Odds ratio

targeted by the United States.[8] These countries could benefit financially from remittances, the transfer of skills and potential investment on migrants' return.[11] However, the source could also experience the disadvantages of a brain drain, resulting in the loss of educational investment and intellectual capital, a reduction in the range of services available and chronic staff shortages in healthcare.[12] In Saudi Arabia, some sponsoring agencies such as the universities are trying to reduce the negative outcome of abroad training by contracting the trainees that they will return to the source country and work there after finishing their training abroad. However, this is not the situation in all governmental agencies who grant the trainees.

The study was subject to some limitations. For example, it included students from only one medical college. Therefore, the results might not be generalizable to students from other institutions. Moreover, small numbers of included subject in this study limited the comparisons between the study groups and the comparison with other studies.

## Conclusion

Most of our individuals intended to study abroad and the destination mainly the USA and Canada. Although Saudi healthcare system experiences a shortage of physicians, some subjects preferred to remain abroad following training. This intention was varied according to gender differences. Therefore, this phenomenon could affect both source countries and destination countries. To identify the dimensions of this phenomenon and its effects on health-care systems, patient care, and physicians' lives further research should be conducted.

### Financial support and sponsorship

Nil.

### Conflicts of interest

There are no conflicts of interest.

## References

1. Gouda P, Kitt K, Evans DS, Goggin D, McGrath D, Last J, *et al.* Ireland's medical brain drain: Migration intentions of Irish medical students. Hum Resour Health 2015;13:11.
2. Imran N, Azeem Z, Haider II, Amjad N, Bhatti MR. Brain drain: Post graduation migration intentions and the influencing factors among medical graduates from Lahore, Pakistan. BMC Res Notes 2011;4:417.
3. Arah OA. The metrics and correlates of physician migration from Africa. BMC Public Health 2007;7:83.
4. Akl EA, Maroun N, Major S, Afif C, Abdo A, Choucair J, *et al.* Post-graduation migration intentions of students of Lebanese medical schools: A survey study. BMC Public Health 2008;8:191.
5. Stilwell B, Diallo K, Zurn P, Dal Poz MR, Adams O, Buchan J, *et al.* Developing evidence-based ethical policies on the migration of health workers: Conceptual and practical challenges. Hum Resour Health 2003;1:8.
6. Syed NA, Khimani F, Andrades M, Ali SK, Paul R. Reasons for migration among medical students from Karachi. Med Educ 2008;42:61-8.
7. Akl EA, Mustafa R, Bdair F, Schünemann HJ. The United States physician workforce and international medical graduates: Trends and characteristics. J Gen Intern Med 2007;22:264-8.
8. Mullan F. The metrics of the physician brain drain. N Engl J Med 2005;353:1810-8.
9. Rao NR, Rao UK, Cooper RA. Indian medical students' views on immigration for training and practice. Acad Med 2006;81:185-8.
10. Moore J, Gale J, Dew K, Simmers D. Student debt amongst junior doctors in New Zealand; Part 2: Effects on intentions and workforce. N Z Med J 2006;119:U1854.
11. Regets MC. Research and Policy Issues in High-Skilled International Migration: A Perspective with Data from the United States. IZA Discussion Paper No. 366. Arlington (VA): National Science Foundation; 2001.
12. Adams O, Kinnon C. A public health perspective. In: International Trade in Health Services: A Developmental Perspective. Geneva: UNCTAD-WHO; 1998.

Original Article

# Gender-Specific Profiles of Cardiovascular Disease in Type 2 Diabetes Mellitus: A Cross-sectional Study

**Reem M. Sallam[1,2,*], Samha M. Z. Alayoubi[3,*], Nasser M. Al-Daghri[3], Alwaleed A. Alhammad[4], Assim A. Alfadda[2,5]**

[1]Department of Medical Biochemistry, Faculty of Medicine, Ain Shams University, Cairo, Egypt, [2]Obesity Research Center, College of Medicine, King Saud University, [3]Department of Biochemistry, College of Science, King Saud University, [4]College of Medicine Research Center, King Saud University, [5]Department of Medicine, College of Medicine, King Saud University, Riyadh, Saudi Arabia

*These authors have equally contributed to this study

## Abstract

**Context:** Cardiovascular disease (CVD) is a chronic macrovascular complication of diabetes mellitus (DM). Factors unique to a group of patients might imply specific differences in the manifestation and/or severity of type 2 DM (T2DM) and CVD. Increasing our knowledge of these factors is critical in designing more robust preventive and/or management approaches for such groups. **Aims:** The aim of this work is to investigate the gender differences among diabetic patients with and without CVD. **Settings and Design:** T2DM patients (64 men and 50 women) were recruited and subdivided according to the presence or absence of CVD as a complication to diabetes. **Subjects and Methods:** Biochemical measurements (glucose, insulin, HbA1c, lipid profile, and liver and kidney function tests), complete blood count, prothrombin and activated partial thromboplastin times, platelet aggregation, tissue factor pathway inhibitor, and plasminogen activator inhibitor-1 (PAI-1) were assessed. Platelet activation was assessed by flow cytometry and aggregation assay. **Statistical Analysis Used:** Microsoft Excel and SPSS were used for data analysis. **Results:** Among the assessed parameters, changes in anthropometry, platelet indices, and PAI-1 were detected. Age, body weight, body mass index, and systolic blood pressure (BP) were significantly higher in women with CVD than in those without. **Conclusions:** The critical association between patients' weight and BP and the development of CVD particularly in diabetic women emphasizes on the need to intensify the efforts for better management of obesity and hypertension specifically among diabetic Saudi women to minimize their CVD risk.

**Keywords:** Cardiovascular disease, diabetes mellitus, female, type 2

## Introduction

Worldwide rise in the prevalence of type 2 diabetes mellitus (T2DM) is parallel to the increasing prevalence of obesity within the general population.[1,2] Moreover, obese individuals with T2DM are at higher risk for diabetic complications than their nonobese counterparts.[3] This phenotype is clearly exemplified in Saudi Arabia and is alarming as it imposes a heavy burden, both from the disease and from its devastating complications and comorbidities.[1,3]

Cardiovascular disease (CVD) is a chronic macrovascular complication of DM.[4] Cellular and molecular factors such as chronic inflammation, cell dysfunction, and tissue damage secondary to chronic hyperglycemia, dyslipidemia, and oxidative stress are among the underlying mechanisms for such vascular complication.[5,6]

Factors unique to a group of patients such as their ethnic background, age group, or gender might imply specific differences in the manifestation and/or severity of chronic noncommunicable diseases including T2DM and CVD.[7] Increasing our knowledge of the molecular bases of these factors will help in designing more robust approach for such groups.

Despite the premenopausal female gender protection against CVD, DM reduces such protection. This may be partly explained by the diabetes-related metabolic changes and consequent shifts in vascular risk profiles.[8] In a recent cross-sectional

**Address for correspondence:** Prof. Reem M. Sallam, Department of Medical Biochemistry, Faculty of Medicine, Ain Shams University, 11381 Abbassia, Cairo, Egypt.
E-mail: reemsallam@med.asu.edu.eg

| Access this article online | |
|---|---|
| Quick Response Code: | Website: www.jnsmonline.org |
| | DOI: 10.4103/JNSM.JNSM_34_18 |



**How to cite this article:** Sallam RM, Z. Alayoubi SM, Al-Daghri NM, Alhammad AA, Alfadda AA. Gender-Specific profiles of cardiovascular disease in type 2 diabetes mellitus: A cross-sectional study. J Nat Sci Med 2018;1:74-81.

study, the risk of developing CVD was increased 2–3 folds in premenopausal diabetic women as compared to their nondiabetic counterpart.[9] The factors responsible for this finding have not been fully investigated. However, several contributing elements such as increased body weight, hypertension, and dyslipidemia are among the conventional factors known to play key roles in the incidence of CVD in general since they are all components of the metabolic syndrome (MetS). Some of these factors, such as waist circumference and high-density lipoprotein-cholesterol (HDL-C), are known to have gender-specific cutoff values.[10,11] However, whether individual components of MetS contribute differently to the precipitation of CVD in men versus women has not been fully assessed. Since the majority of cardiovascular events are precipitated by vessel occlusion caused by thrombus on a ruptured atherosclerotic plaque, platelet dysfunction in T2DM patients may contribute to their increased CVD risk.[12-14] Likewise, factors contributing to maintaining the blood vessels' integrity and patency such as fibrinolysis and endothelial cell function may need further investigation regarding possible gender differences.[15]

The present work is designed to be a pilot study aiming to investigate the gender difference's influence in a group of adult T2DM Saudi men and women, both without and with CVD.

## Subjects and Methods

This is a cross-sectional study. General medical information was extracted from the archived patients' files. Ethical approval from the Institutional Review Board was obtained before the study. Informed consent was obtained from all individual participants included in the study. One hundred and fourteen adult Saudi patients with T2DM were recruited (males: $n$ = 64, age range: 20–70 years old, average age ± standard deviation [SD]: 46.5 years old ± 9.4 and females: $n$ = 50, age range: 16–80 years old, average age ± SD: 48.9 years old ± 12.4). Inclusion criteria included adult age, Saudi nationality, and T2DM, with or without CVD. CVD was defined as a history of coronary heart disease manifested by myocardial infarction, angina pectoris, or heart failure; cerebral ischemia manifested by stroke or transient ischemic attack; or peripheral arterial disease manifested by intermittent claudication. Exclusion criteria included acute chest pain, renal failure, pregnancy, liver diseases, and consumption of medications that could affect the coagulation or fibrinolytic systems.

Participants were divided into two groups based on gender. Each group was further subdivided according to the presence of CVD as a complication to T2DM: (T2DM men without complications [$n$ = 28], T2DM men with CVD complications [$n$ = 36], T2DM women without complications [$n$ = 32], and T2DM women with CVD complications [$n$ = 18]). Fasting venous blood was drawn and processed taking precautions that avoid platelet activation. Ethylenediaminetetraacetic acid-whole blood was used for flow cytometry and for the complete blood count (CBC). Citrated whole blood was used to assess platelet aggregation, and citrated plasma was used to determine prothrombin time (PT) and activated partial thromboplastin time (APTT). The remaining citrated plasma was stored at −80° C for enzyme-linked immunosorbent assay (ELISA). Serum was used for biochemical measurements including fasting glucose (FG), fasting insulin, and HbA1c. Homeostasis model assessment for insulin resistance (HOMA-IR) was calculated.[16] Lipid profile including total cholesterol, triacylglycerol (TG), low-density lipoprotein-cholesterol (LDL-C), and HDL-C; liver function tests including aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transferase (GGT), alkaline phosphatase (ALP), bilirubin, total protein, and albumin; and renal function tests including serum creatinine and blood urea nitrogen were all assessed. The creatinine clearance was estimated by applying Cockroft-Gault formula.[17] The biochemical tests were performed using the Dimension Xpand plus autoanalyzer (Siemens Healthcare Diagnostics, USA). Insulin was measured by electrochemiluminescence using a Cobas e411 immunoanalyzer (Roche, USA).

Hemostatic and fibrinolytic parameters, in addition to measuring the platelet aggregation function and the platelet cell surface expression of activation markers, were assessed. Tests were performed within 3 h after venipuncture. Adenosine diphosphate (ADP; from Chrono-log, final concentration 10 μmol/L)-induced platelet aggregation in whole blood was assessed using a Chrono-Log aggregometer (Model 570VS equipped with Chrono-log AGGRO/LINK® Software). Changes in electrical impedance in ohms (Ω) was quantified.[18]

Platelet surface expression of activation markers using flow cytometry was performed according to the manufacturer's instructions. All reagents and monoclonal antibodies (MoAbs) were from BD (USA). Lysis of red blood cells (RBCs) and labeling of cell surface receptors on the platelets and monocytes were performed. MoAbs used included: FITC-conjugated CD63 (for activation-dependent platelet membrane protein), PerCP-conjugated CD61 (for constitutively expressed platelet membrane protein), and PE-conjugated CD14 (for constitutively expressed monocyte membrane protein). Equal concentrations of isotype-matched similarly conjugated nonimmune mouse IgG were used as negative control. Following cell labeling and fixation, using 2% paraformaldehyde, platelets were identified by flow cytometry based on their light scatter properties and immunostaining with anti-CD61 MoAb. Activated platelets were then quantified by assessing the percent platelets immunostained with anti-CD63 MoAb. Monocytes were identified by their light-scatter properties and immunostaining with anti-CD14 MoAb. Platelet-monocyte aggregates were identified by their double immunostaining with both anti-CD14 and anti-CD61 MoAbs.

Circulating levels of tissue factor pathway inhibitor both total and free (TFPI; total and free) and of plasminogen activator inhibitor-1 (PAI-1) were measured in citrated

plasma using ELISA (Diagnostica Stago, France) following the manufacturer's instructions. The intra- and inter-assay coefficients of variation for the ELISA kits used were as follows; total TFPI kit: <5 and <8%, free TFPI kit: <6 and <10%, and PAI-1 kit: 6.53 and 8.69%, respectively.

Microsoft Excel and IBM SPSS Statistics for Windows, Version 22.0. (Armonk, NY: IBM Corp) were used to compare both groups and subgroups. Central values and the data spread were described as mean ± SD and/or standard error of the mean. Student's *t*-test or Mann–Whitney U-test was used to compare groups. Spearman's rank correlation coefficient or Spearman's rho was used to determine correlation coefficient between various variables. $P \leq 0.05$ was considered statistically significant.

## Results

Analysis of the anthropometric, biochemical, and hematological data of the recruited T2DM patients is demonstrated in Table 1. Both men and women subjects were of matching age. Although there was a trend for a higher average body weight among men, the average body mass index (BMI) values in both genders were matching. Similarly, blood pressure (BP) average values were comparable in both genders. Biochemical measurements analysis has demonstrated no difference in FG or fasting insulin levels among both genders. On the other hand, men were on average more insulin resistant than women as indicated by the HOMA-IR index. Insulin resistance indices showed a wide dispersion of values in both genders as apparent from the large SD values. Both genders were on average maintaining more or less acceptable glycemic control for T2DM patients as demonstrated by the HbA1c%. Similarly, the lipid measures have demonstrated no differences among both genders. Significantly lower erythrocyte parameters were demonstrated in women relative to men. On the other hand, no intergender significant differences were detected in total white blood cells' count, platelet count and volume, or PT and APTT.

Analyzing the participant's anthropometric data after categorizing them according to the presence or absence of CVD has demonstrated gender-related and CVD-related differences as shown in Tables 2 and 3. Within both genders, the average age was significantly higher in the group with CVD complications as compared to the group with no complications. While only within the women group, the body weight, BMI, and systolic BP (SBP) were significantly higher in the presence of CVD.

Among the T2DM women without CVD complication, 53% were premenopausal, while 47% were postmenopausal [Figure 1a], 31% had SBP >135 mmHg, and none had diastolic BP (DBP) ≥85 mmHg. On the other hand, in the group of T2DM women with CVD, 27% were premenopausal, while 73% were postmenopausal [Figure 1b], 44% of this group had SBP >135 mmHg, and 17% had DBP ≥85 mmHg.

Analyzing fasting insulin and glucose, HOMA-IR, HBA1c, and lipid profile after categorizing the recruited participants, according to the presence or absence of CVD, has not revealed any significant difference in both genders. However, among these measures, the presence of CVD was associated with a lower trend in HDL-C average level and a higher trend in TG level ($P > 0.05$) in the group of T2DM women [Figure 2]. A significant positive correlation was detected between insulin level and TG level in T2DM women (when women with

**Table 1: Anthropometric, biochemical, and hematological parameters of type 2 diabetes mellitus men and women**

| Parameter | Mean±SD | | *P* |
|---|---|---|---|
| | Men (*n*=64) | Women (*n*=50) | |
| Age (years) | 46.5±9.4 | 48.9±12.4 | 0.25 |
| Weight (kg) | 88.4±16.1 | 82.17±17.2 | 0.06 |
| BMI (kg/m$^2$) | 32.01±10.96 | 33.85±7.21 | 0.32 |
| Systolic BP (mmHg) | 133.08±15.97 | 131.74±18.9 | 0.69 |
| Diastolic BP (mmHg) | 75.4±14.08 | 74.7±8.59 | 0.76 |
| Fasting glucose (mmol/L) | 10.2±3.9 | 8.98±3.23 | 0.075 |
| Fasting insulin (µIU/ml) | 9.79±12.45 | 11.73±15.18 | 0.60 |
| HOMA-IR | 4.53±6.86 | 1.69±5.61 | **0.019*** |
| HbA1c (%) | 8.13%±2.0 | 7.64%±1.61 | 0.22 |
| Cholesterol (mmol/L) | 4.63±3.83 | 4.56±0.69 | 0.91 |
| HDL-C (mmol/L) | 1.37±0.44 | 1.46±0.75 | 0.49 |
| LDL-C (mmol/L) | 2.58±0.72 | 2.75±0.61 | 0.33 |
| Triacylglycerol (mmol/L) | 1.87±1.06 | 1.60±0.95 | 0.17 |
| GGT (U/L) | 51.1±36.09 | 42.07±46.37 | 0.30 |
| ALP (U/L) | 82.94±27.39 | 94.57±28.69 | **0.03*** |
| AST (U/L) | 22.11±10.66 | 20.53±11.39 | 0.45 |
| ALT (U/L) | 27.41±6.95 | 39.69±16.48 | **<0.001***** |
| WBC (×10$^3$/µl) | 6.71±1.91 | 7.39±1.76 | 0.067 |
| RBC (×10$^6$/µl) | 5.02±0.54 | 4.68±0.47 | **0.001***** |
| Hgb (g/dl) | 14.61±1.38 | 13.02±1.52 | **<0.001***** |
| HCT (%) | 42.78±3.79 | 38.62±3.92 | **<0.001***** |
| MCV (fl) | 85.71±6.63 | 82.75±7.18 | **0.037*** |
| MCH (pg) | 29.32±2.59 | 27.91±2.94 | **0.014*** |
| MCHC (g/dl) | 34.18±0.76 | 33.67±0.94 | **0.004**** |
| RDW (%) | 13.61±1.05 | 14.53±1.64 | **0.002**** |
| PLT (×10$^3$/µl) | 267.21±60.8 | 293.82±85.61 | 0.08 |
| MPV (fl) | 9.2±1.1 | 9.47±1.0 | 0.216 |
| PT (s) | 13.94±0.92 | 14.1±3.01 | 0.741 |
| APTT (s) | 36.39±4.1 | 36.54±4.2 | 0.867 |

*P* value is significant at ≤0.05 and is adjusted for age and BMI. Significant *P* values are in bold. *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$. SD: Standard deviation. BMI: Body mass index, BP: Blood pressure, HOMA-IR: Homeostasis model assessment for insulin resistance, HbA1c: Glycated haemoglobin, HDL-C: High density lipoprotein-cholesterol, LDL-C: Low density lipoprotein-cholesterol, GGT: γ-Glutamyl transferase, ALP: Alkaline phosphatase, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, WBC: White blood cells, RBC: Red blood cells, Hgb: Hemoglobin, HCT: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration; RDW: Red cell distribution width, PLT: Platelet count, MPV: Mean platelet volume, PT: Prothrombin time, APTT: Activated partial thromboplastin time

**Table 2: Anthropometric parameters of type 2 diabetes mellitus men and women, without and with cardiovascular disease**

| Parameter | Men, mean±SD (range) | | | Women, mean±SD (range) | | |
|---|---|---|---|---|---|---|
| | Without CVD (*n*=28) | With CVD (*n*=36) | *P* | Without CVD (*n*=32) | With CVD (*n*=18) | *P* |
| Age (years) | 43.57±10.49 (20-55) | 48.81±8.06 (30-70) | **0.027*** | 46.1±13.71 (16-80) | 53.56±8.36 (32-69) | **0.044*** |
| Weight (kg) | 88.78±15.07 (60-133) | 89.66±17.01 (59-126) | 0.48 | 76.72±14.47 (40.5-109) | 90.94±17.99 (63.4-128) | **0.005**** |
| BMI (kg/m$^2$) | 29.75±4.68 (21.3-42.0) | 33.78±13.86 21.9-42.8 | 0.15 | 31.4±5.99 (18.3-44.8) | 37.68±7.44 (27.4-59.2) | **0.003**** |
| Systolic BP (mmHg) | 129.29±14.9 (101-164) | 136.03±16.35 (109-190) | 0.094 | 126.69±17.26 (95-164) | 139.89±19.12 (103-185) | **0.023*** |
| Diastolic BP (mmHg) | 74.16±18.81 (65-95) | 76.39±9.02 (58-102) | 0.535 | 73.93±6.78 (60-90) | 75.94±11.02 (60-100) | 0.441 |

*P* value is significant at ≤**0.05** and is adjusted for age and BMI. Statistically significant *P* values are in bold. *$P \leq 0.05$, **$P \leq 0.01$. CVD: Cardiovascular disease, SD: Standard deviation, BMI: Body mass index, BP: Blood pressure

**Table 3: Percentage of type 2 diabetes mellitus women in each of the World Health Organization bodyweight category**

| WHO bodyweight category† | T2DM women without CVD (%) | T2DM women with CVD (%) |
|---|---|---|
| Lean | 11 | - |
| Overweight | 18 | 11 |
| Obese | 71 | 89 |

†According to WHO criteria of obesity based on BMI: Lean: BMI=18.5-24.9 kg/m$^2$, Overweight: BMI=25-29.9 kg/m$^2$, and Obese: BMI ≥30 kg/m$^2$. WHO: World Health Organization, T2DM: Type 2 diabetes mellitus, CVD: Cardiovascular disease, BMI: Body mass index

and without CVD were combined in one group) (correlation coefficient (ρ) = 0.586, and *P* = 0.02). This was not detected in T2DM men group. A positive trend was maintained between insulin and TG levels in T2DM women with CVD, although it did not reach statistical significance.

Analysis of liver enzymes measurements as liver function tests has shown that T2DM women had significantly higher levels of circulating ALP and ALT than T2DM men [Table 1 and Figure 3]. Within the same gender, the occurrence of CVD was not associated with a statistically significant difference in liver function tests (*P* > 0.05) [Figure 4] or in kidney function tests (data not shown).

Only T2DM men demonstrated CVD-related significant change in the hemoglobin concentration and hematocrit value, both were significantly lower in T2DM men with CVD relative to those without CVD (*P* = 0.026 and *P* = 0.042, respectively).

The hemostatic parameters, PT and APTT, assessed revealed no gender-related or CVD-related statistical significant differences [Table 1].

The CBC showed a trend of higher platelet count in T2DM women relative to T2DM men, although this trend did not reach statistical significance [Table 1]. However, the platelet count difference was more apparent using the flow cytometric cell surface expression of CD61, where the average ± SD of the percentage of CD61-positive particles was 19.87% ± 15.85 (men) and 28.03% ± 16.14 (women) (*P* = 0.019).

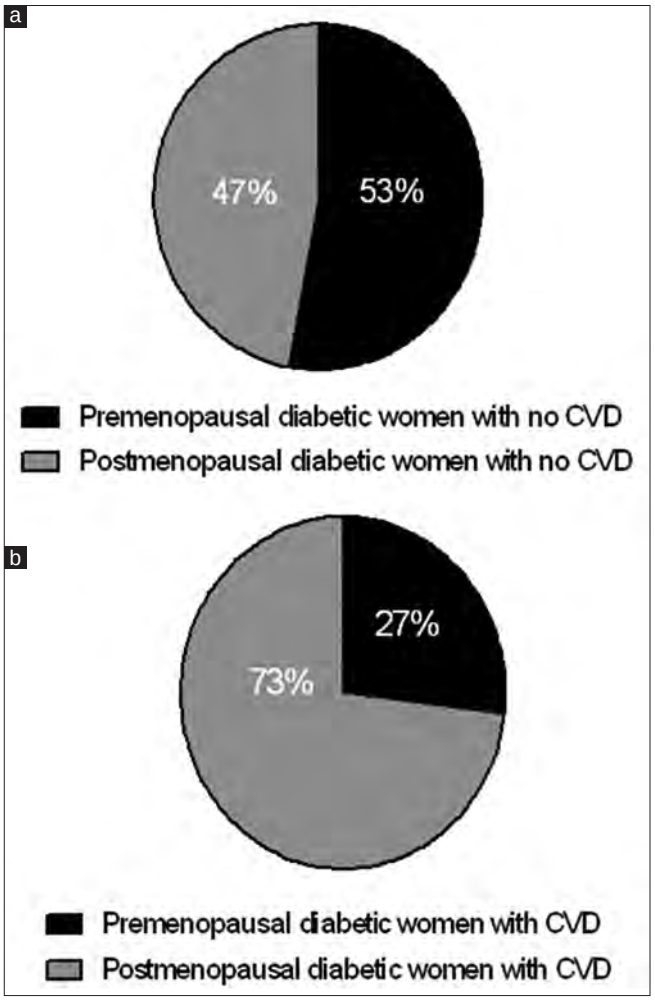


**Figure 1:** Percentage of pre- and post-menopausal type 2 diabetes mellitus women with no cardiovascular disease (a) and with cardiovascular disease (b)

There was a significant difference in platelet count only in female group categorized according to the presence of CVD, where lower platelets' count was found in T2DM female with CVD as compared to those without CVD (percentage of CD61-positive particles was 33.3% ±16.1 [without CVD] and 18.1% ±16.2 [with CVD] *P* = 0.016).

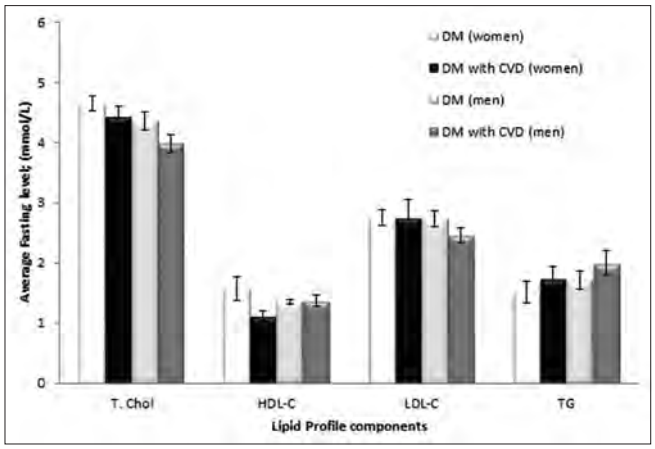


**Figure 2:** Fasting lipid profile in diabetes mellitus men and women with or without cardiovascular disease. Data are presented as mean ± standard error of the mean (SEM). SEM values are shown as vertical error bars. Abbreviations used: DM: diabetic; T. Chol: Total cholesterol; HDL-C: High-density lipoprotein-cholesterol; LDL-C: Low-density lipoprotein-cholesterol; TG: Triacylglycerol

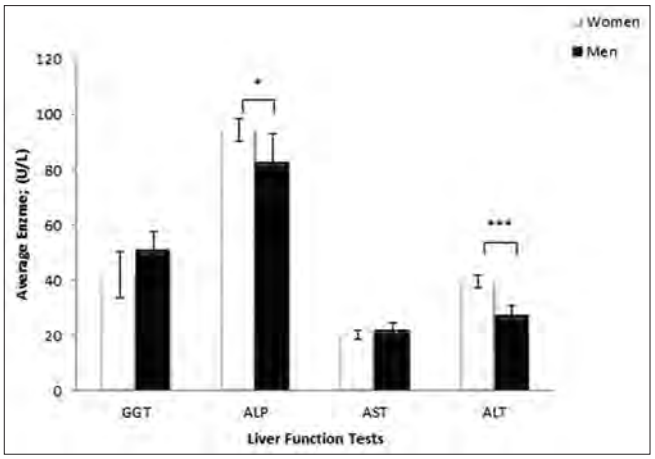


**Figure 3:** Liver function tests in diabetes mellitus men and women. Data are presented as mean ± standard error of the mean (SEM). SEM values are shown as vertical error bars. Student *t*-test significance: $*P \leq 0.05$, $***P \leq 0.001$. GGT: γ-glutamyl transferase; ALP: Alkaline phosphatase; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase

No statistically significant difference was observed in the ADP-induced platelet aggregation, basal activation level of circulating platelets by flow cytometry, and the ELISA measurements of the hemostatic parameter (total and free TFPI) (data not shown). Circulating levels of PAI-1, on the other hand, showed a trend of increasing levels among T2DM men with CVD as compared to those without CVD ($P = 0.08$). This was not observed among T2DM women. Significant positive correlation between PAI-1 and body weight was detected among the group of all T2DM patients (men and women combined) having CVD ($\rho = 0.396$, $P = 0.045$). No such correlation was found when we analyzed the T2DM men and women separately nor when we subcategorized each gender based on the presence of CVD. PAI-1 levels in all T2DM patients (both genders and regardless of the presence or absence of CVD) were positively correlated with the BMI ($\rho = 0.258$, $P = 0.05$). Interestingly, only in T2DM women group (regardless of the presence or absence of CVD) circulating PAI-1 levels positively correlate with fasting insulin ($\rho = 0.867$, $P < 0.001$), with HOMA-IR ($\rho = 0.860$, $P < 0.001$), and with fasting TG ($\rho = 0.360$, $P = 0.024$).

## Discussion

The present work demonstrates gender-related differences in certain parameters in T2DM patients when assessing the insulin resistance index, liver enzymes, RBCs parameters, and platelets count. Only in T2DM women was there a significant positive correlation between fasting insulin and TG and between PAI-1 levels and each of the following parameters: fasting insulin, HOMA-IR, and TG. Both genders showed CVD-related significant difference in age, where older age is more associated with the occurrence of CVD in T2DM. T2DM women, in particular, showed CVD-related significant differences in their body weight, BMI, and SBP.

Intergender differences in platelets count and volume were previously demonstrated in healthy population where higher platelets count and lower mean platelet volume were reported in healthy women as compared to healthy men.[19] In our study, an association between platelet count and the presence of CVD among T2DM women was demonstrated by the significant decrease in the percentage of particles positive for CD61 in the presence of CVD. Although this finding needs further confirmation on a larger sample size, a possible explanation might be the increased consumption of platelets due to the thrombotic events. Platelet aggregation, on the other hand, was not affected in the group of T2DM patients with CVD; a finding that might be related to the observed large interindividual variation in platelets' aggregation response. It is not uncommon to find conflicting results in the literature in this regard, i.e., the correlation between platelet aggregation response and platelet indices.[19] Such ambiguity in describing platelet contribution to the occurrence of CVD in T2DM seems to be the result of the complex nature of the platelets' activity, and their integration with other factors involved in maintaining normal blood flow. We suggest that it is the balance between two states; namely platelet responsiveness upon activation and platelet's basal-state of activity; that leads the T2DM patient to manifest specific vascular complication versus another. Additional complexity possibly exists, namely the interindividual variation, not only in platelets count and functions but also in various other hemostatic/thrombotic factors. Results demonstrated in the current work indicate that measuring both platelet indices (count and volume) and platelet function, perhaps by more than one approach; for instance, measuring platelet microparticles' level and activity,[20] or assessing activation-induced intraplatelet calcium mobilization, in addition to platelet aggregation; in larger cohort may provide a better understanding of the platelet status in T2DM.

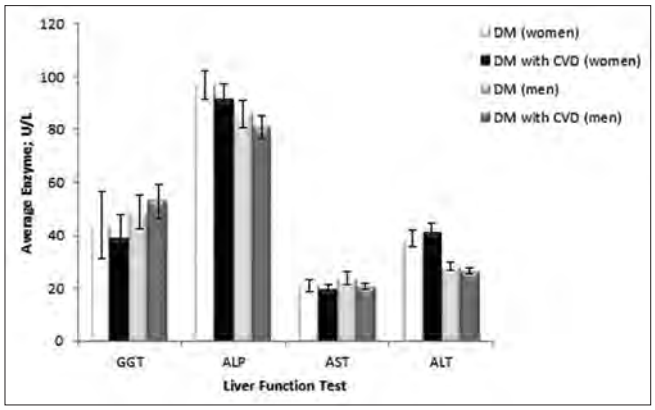


**Figure 4:** Liver function tests in diabetes mellitus men and women with or without cardiovascular disease. Data are presented as mean ± standard error of the mean (SEM). SEM values are shown as vertical error bars. GGT: g-glutamyl transferase; ALP: Alkaline phosphatase; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase

Impaired fibrinolysis, as detected in the present work as well as in others,[21] signals a hazardous situation with high risk of precipitating thrombovascular complications in uncontrolled T2DM patients at any time point, especially in those patients with other risk factors as dyslipidemia and atherosclerosis. Factors such as hyperinsulinemia and obesity might partially contribute to the impaired fibrinolysis. A few years ago, Stegenga *et al*. have demonstrated that hyperinsulinemia inhibits fibrinolysis irrespective of glucose concentration and that hyperglycemia stimulates coagulation irrespective to insulin concentration.[21] This is in line with our findings of significant positive correlation between PAI-1, which is not only a marker for the fibrinolytic system activity but also for inflammation and for endothelial dysfunction and both fasting insulin concentrations and insulin resistance index in T2DM. This correlation seems to be contributed more by the female group of T2DM patients, since the significant association observed in the whole T2DM group becomes statistically more significant when the female group was separately analyzed, while the association becomes no longer significant when the male group was separately analyzed. Since the majority of the circulating PAI-1 is contributed by adipose tissue, obese patients are expected to have higher levels of PAI-1.[22,23] Indeed, results obtained from the present study confirm this and show a strong association between obesity measures (body weight and BMI) and circulating levels of PAI-1. This association is found to be related to the presence of diabetic complications but is not gender related. Alternatively, based on a study showing that high PAI-1 levels predicted the risk of T2DM and that this predictive ability extended after a long-term follow-up period of 9 years,[24] one can postulate that, in a population at risk of developing diabetes (such as the offspring of T2DM), elevated PAI-1 level may be an independent marker for predicting the disease. Further studies are needed to test this postulation.

CVD is uncommon in premenopausal women, particularly in the absence of other risk factors.[25] This is confirmed in the present study, and it highlights the protective roles played by the female reproductive hormonal profile and the favorable lipid profile in the premenopausal period. This might also reflect the unfavorable inflammatory milieu of obesity, since T2DM women with CVD were on average more obese, and hence more exposed to the inflammatory cytokines/adipokines secreted by the adipose tissue than those without the CVD complications.[26]

Insulin resistance, DM, and dyslipidemia are key components of the MetS.[27] In accordance with data reported by Jax *et al*.,[28] who have shown a statistically significant positive correlation between fasting TG – while not total cholesterol nor HDL-C – and fasting insulin levels in T2DM complicated with CVD, our data demonstrated similar finding among T2DM women both controlled and complicated. A positive trend was maintained between insulin and TG levels in the group of T2DM women with CVD complications, although it did not reach statistical significance. This might be explained by the natural history of T2DM. The appearance of complications represents a late stage in the disease progress, where the pancreas is unable to compensate for the peripheral insulin resistance by secreting more insulin.[29] Nevertheless, the association between CVD as a macrovascular complication of T2DM and both hyperglycemia and hyperinsulinemia is not fully understood, and recent evidence suggests that it is the peripheral insulin resistance state that increases the risk for CVD rather than the insulin level *per se*. Hyperinsulinemia and insulin resistance are also key factors in the mechanism of atherosclerosis and hence have been the focus of intense studies for almost four decades. Several research groups have demonstrated that elevated fasting plasma insulin levels were strongly linked to enhanced atherosclerosis, and that they are associated with hypertension and obesity, both are risk factors for CVD. These studies were recently analyzed in a systematic review by Kelly *et al*.[30]

Our results are consistent with the general consensus that patients with T2DM, who also manifest the MetS or its main components carry a higher risk of CVD complications than those who have T2DM alone and are, otherwise well-controlled.[31] The link between various T2DM-associated complications/comorbidities includes dietary factors, metabolic, endothelial/vascular dysfunction, and kidney diseases leading to sodium retention, glomerular hyperfiltration, and proteinuria.[32,33] Moreover, recent large cohort prospective studies have demonstrated that obesity is strongly associated with an increased risk of T2DM, hypertension, and dyslipidemia,[34] and that it is a strong and independent predictor of death from CVD among women.[35,36] In fact, studies have confirmed that there is no healthy pattern of increased weight and that on the long term, even the recently described category of "obese but metabolically healthy" individuals are still at an increased risk of CVD and death from any cause, relative to "lean and metabolically healthy" individuals. This was recently published in systematic reviews and meta-analyses.[37,38] The current work supports this notion

and emphasizes that it is critically important to control body weight, especially among women suffering from T2DM.

Certain interesting findings reported in the current work are worthy for targeting in future studies. For instance, a liver imaging technique looking at the prevalence of fatty liver among diabetic female with and without CVD, as opposed to male participants might explain the intergender difference in the liver function tests reported in the present study. In addition, measuring the hemoglobin and hematocrit values in a larger cohort of diabetic population of both genders in the presence or absence of cardiovascular complications might help in better understanding for related finding of the present work. This can also be related to recent works that demonstrated a strong association between RBC indices and CVD.[39,40]

The present study has strength points and some limitations. One limitation is imposed by the small sample size. Using the BMI as the obesity marker, as it represents total body weight and not specifically fat mass and central obesity, may be considered a limitation. However, this was based on the general consensus that if BMI is ≥30 kg/m$^2$, central obesity can be assumed and measures of central obesity need not to be measured.[31] Further limitation was due to the performed platelets' function assessment method, namely the ADP-induced platelet aggregation, which although informative, has shown a wide individual variation. Increasing the sample size and using an additional platelet functional assessment method is suggested for future analysis. On the other hand, focusing on confirmed cases of T2DM without and with CVD complication among men and women and highlighting the factors differentiating these subgroups are clearly strength points. The conductance of this study among Saudi adult men and women was meant to obtain meaningful pilot study data for a population that is not well-studied yet, allowing for assessing ethnicity- and/or gender-related characteristic in future studies. This is of particular significance in parameters such as circulating level of PAI-1 which was recently reported to manifest ethnicity-related differences.[23,41]

## Conclusions

Results of the current study demonstrated several gender- and CVD-related differences among adult T2DM patients. Obesity, impaired fibrinolysis, and platelet count represent factors associated with the presence of cardiovascular complication, particularly among T2DM women. A link between PAI-1, obesity, insulin resistance, and atherogenic lipid profile among T2DM is also demonstrated. Further studies are needed to better understand the altered thrombotic milieu. Stringent control of obesity and BP is critical in the management of T2DM patients in general and among women in particular.

### Financial support and sponsorship

This study was financially supported by King Abdul-Aziz City for Science and Technology (grant # LGP-12-9) and The Strategic Technologies Program of the National Plan for Sciences and Technology and Innovation in Kingdom of Saudi Arabia (Grant No. 08-MED 513-02 and Grant No. 11-MED1907-02).

### Conflicts of interest

There are no conflicts of interest.

## References

1. Scheen AJ, Van Gaal LF. Combating the dual burden: Therapeutic targeting of common pathways in obesity and type 2 diabetes. Lancet Diabetes Endocrinol 2014;2:911-22.
2. Bhupathiraju SN, Hu FB. Epidemiology of obesity and diabetes and their cardiovascular complications. Circ Res 2016;118:1723-35.
3. Riobó Serván P. Obesity and diabetes. Nutr Hosp 2013;28 Suppl 5:138-43.
4. Donaghue KC, Chiarelli F, Trotta D, Allgrove J, Dahl-Jorgensen K. Microvascular and macrovascular complications associated with diabetes in children and adolescents. Pediatr Diabetes 2009;10 Suppl 12:195-203.
5. Bornfeldt KE. 2013 Russel Ross memorial lecture in vascular biology: Cellular and molecular mechanisms of diabetes mellitus-accelerated atherosclerosis. Arterioscler Thromb Vasc Biol 2014;34:705-14.
6. Varga ZV, Giricz Z, Liaudet L, Haskó G, Ferdinandy P, Pacher P, *et al.* Interplay of oxidative, nitrosative/nitrative stress, inflammation, cell death and autophagy in diabetic cardiomyopathy. Biochim Biophys Acta 2015;1852:232-42.
7. Sliwa K, Acquah L, Gersh BJ, Mocumbi AO. Impact of socioeconomic status, ethnicity, and urbanization on risk factor profiles of cardiovascular disease in Africa. Circulation 2016;133:1199-208.
8. Dantas AP, Fortes ZB, de Carvalho MH. Vascular disease in diabetic women: Why do they miss the female protection? Exp Diabetes Res 2012;2012:570598.
9. García NH, Pérez HA, Spence JD, Armando LJ. Risk of vascular disease in premenopausal women with diabetes mellitus. Clin Ther 2014;36:1924-34.
10. World Health Organization. Waist Circumference and Waist-Hip Ratio: Report of a WHO Expert Consultation. 8-11 December, 2008. Geneva: World Health Organization; 2011.
11. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, *et al.* Harmonizing the metabolic syndrome: A joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation 2009;120:1640-5.
12. Webb DR, Gray LJ, Khunti K, Campbell S, Dallosso H, Davies MJ, *et al.* Contrasting cardiovascular risk profiles and prescribed cardio-protective therapies in newly-diagnosed type 2 diabetes identified through screening and standard practice. Diabetes Res Clin Pract 2011;91:280-5.
13. Badimon L, Padró T, Vilahur G. Atherosclerosis, platelets and thrombosis in acute ischaemic heart disease. Eur Heart J Acute Cardiovasc Care 2012;1:60-74.
14. Neergaard-Petersen S, Hvas AM, Kristensen SD, Grove EL. Platelets and antiplatelet therapy in patients with coronary artery disease and diabetes. Semin Thromb Hemost 2016;42:234-41.
15. Mylotte D, Kavanagh GF, Peace AJ, Tedesco AF, Carmody D, O'Reilly M, *et al.* Platelet reactivity in type 2 diabetes mellitus: A comparative analysis with survivors of myocardial infarction and the role of glycaemic control. Platelets 2012;23:439-46.
16. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC, *et al.* Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412-9.
17. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976;16:31-41.
18. Sweeney JD, Hoernig LA, Fitzpatrick JE. Whole blood aggregation in von Willebrand disease. Am J Hematol 1989;32:190-3.
19. Park BJ, Shim JY, Lee HR, Jung DH, Lee JH, Lee YJ, *et al.* The relationship of platelet count, mean platelet volume with metabolic syndrome according to the criteria of the American Association of Clinical Endocrinologists: A focus on gender differences. Platelets

2012;23:45-50.
20. Zhang X, McGeoch SC, Johnstone AM, Holtrop G, Sneddon AA, MacRury SM, *et al.* Platelet-derived microparticle count and surface molecule expression differ between subjects with and without type 2 diabetes, independently of obesity status. J Thromb Thrombolysis 2014;37:455-63.
21. Stegenga ME, van der Crabben SN, Blümer RM, Levi M, Meijers JC, Serlie MJ, *et al.* Hyperglycemia enhances coagulation and reduces neutrophil degranulation, whereas hyperinsulinemia inhibits fibrinolysis during human endotoxemia. Blood 2008;112:82-9.
22. Alessi MC, Juhan-Vague I. Metabolic syndrome, haemostasis and thrombosis. Thromb Haemost 2008;99:995-1000.
23. Barnard SA, Pieters M, De Lange Z. The contribution of different adipose tissue depots to plasma plasminogen activator inhibitor-1 (PAI-1) levels. Blood Rev 2016;30:421-9.
24. Alessi MC, Nicaud V, Scroyen I, Lange C, Saut N, Fumeron F, *et al.* Association of vitronectin and plasminogen activator inhibitor-1 levels with the risk of metabolic syndrome and type 2 diabetes mellitus. Results from the D.E.S.I.R. prospective cohort. Thromb Haemost 2011;106:416-22.
25. Ostadal B, Ostadal P. Sex-based differences in cardiac ischaemic injury and protection: Therapeutic implications. Br J Pharmacol 2014;171:541-54.
26. Khera A, Vega GL, Das SR, Ayers C, McGuire DK, Grundy SM, *et al.* Sex differences in the relationship between C-reactive protein and body fat. J Clin Endocrinol Metab 2009;94:3251-8.
27. Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. Diabetes 1988;37:1595-607.
28. Jax TW, Peters AJ, Plehn G, Schoebel FC. Relevance of hemostatic risk factors on coronary morphology in patients with diabetes mellitus type 2. Cardiovasc Diabetol 2009;8:24.
29. Ferrannini E, Mari A. B-cell function in type 2 diabetes. Metabolism 2014;63:1217-27.
30. Kelly CT, Mansoor J, Dohm GL, Chapman WH 3rd, Pender JR 4th, Pories WJ, *et al.* Hyperinsulinemic syndrome: The metabolic syndrome is broader than you think. Surgery 2014;156:405-11.
31. Eckel RH, Alberti KG, Grundy SM, Zimmet PZ. The metabolic syndrome. Lancet 2010;375:181-3.
32. DeMarco VG, Aroor AR, Sowers JR. The pathophysiology of hypertension in patients with obesity. Nat Rev Endocrinol 2014;10:364-76.
33. Domingueti CP, Dusse LM, Carvalho MD, de Sousa LP, Gomes KB, Fernandes AP, *et al.* Diabetes mellitus: The linkage between oxidative stress, inflammation, hypercoagulability and vascular complications. J Diabetes Complications 2016;30:738-45.
34. Schmiegelow MD, Andersson C, Køber L, Andersen SS, Norgaard ML, Jensen TB, *et al.* Associations between body mass index and development of metabolic disorders in fertile women – A nationwide cohort study. J Am Heart Assoc 2014;3:e000672.
35. van Dam RM, Li T, Spiegelman D, Franco OH, Hu FB. Combined impact of lifestyle factors on mortality: Prospective cohort study in US women. BMJ 2008;337:a1440.
36. Bruce SA. The association between central fat distribution and recurrent cardiovascular disease events in female survivors of nonfatal myocardial infarction. J Cardiovasc Nurs 2015;30:E15-22.
37. Kramer CK, Zinman B, Retnakaran R. Are metabolically healthy overweight and obesity benign conditions? A systematic review and meta-analysis. Ann Intern Med 2013;159:758-69.
38. Lin H, Zhang L, Zheng R, Zheng Y. The prevalence, metabolic risk and effects of lifestyle intervention for metabolically healthy obesity: A systematic review and meta-analysis: A PRISMA-compliant article. Medicine (Baltimore) 2017;96:e8838.
39. Tziakas D, Chalikias G, Grapsa A, Gioka T, Tentes I, Konstantinides S, *et al.* Red blood cell distribution width: A strong prognostic marker in cardiovascular disease: Is associated with cholesterol content of erythrocyte membrane. Clin Hemorheol Microcirc 2012;51:243-54.
40. Mallikethi-Reddy S, Briasoulis A, Akintoye E, Afonso L. Novel biomarkers with potential for cardiovascular risk reclassification. Biomarkers 2017;22:189-99.
41. Kumari B, Srivastava S, Chatterjee T, Vardhan R, Tyagi T, Gupta N, *et al.* Study of associated genetic variants in Indian subjects reveals the basis of ethnicity related differences in susceptibility to venous thromboembolism. Thrombosis 2014;2014:182762.

Brief Report

# Cerebral Injury in Diabetic Ketoacidosis: Is there a Room for Conservative Management?

**Riad A. Sulimani, Anwar A. Jammah, Ibrahim M. Ghozzi[1], Hadil A. Alotair[2], Suleiman A. Al-Mohaya[1], Tarek E. Ashour[3]**

Departments of Medicine and [2]Critical Care Medicine, College of Medicine, King Saud University, [1]Department of Medicine, Security Forces Hospital, Riyadh, Saudi Arabia, [3]Department of Medicine, New Mowasat Hospital, Hawalli, Kuwait

## Abstract

Diabetic ketoacidosis is a manifestation of decompensated glycemic control. Two cases are outlined, representing severe cerebral edema in one case and multiple cerebral infarcts in the other. Favorable outcome was achieved in both cases with conservative management, excluding the immediate use of mannitol in the first patient and the use of thrombolytic or full anticoagulant therapy in the second.

**Keywords:** Diabetic ketoacidosis, cerebral edema, mannitol, thrombolysis

## Introduction

Cerebral crisis during diabetic ketoacidosis (DKA) is a serious complication with significant morbidity and mortality.[1,2] Cerebral edema (CE) alone is responsible for 50%–80% of diabetes-related deaths.[1,2] Brain injury with swelling and lateral ventricle compression appears to be common in DKA but often subclinical.[3] In addition to CE, up to 10% of cerebral complications in DKA may be related to hemorrhagic or ischemic strokes.[4] Guidelines and recommendations for the management of CE have been made, including the early use of mannitol during management.[5] In the case of ischemic stroke, there is still some controversy whether thrombolytic treatment should be offered to patients.[6] In this report, two patients with excellent outcomes are reported: one with severe CE with no mannitol given initially and a patient with multiple ischemic lesions with no antithrombolytic or therapeutic anticoagulant therapy were given.

## Case Reports

### Case 1

A 13-year-old boy (weight = 40 kg) with Type 1 diabetes mellitus was referred to our hospital from another hospital 12 h after presentation. In the previous hospital, he was a conscious state but with severe DKA (pH of 6.93, bicarbonate 7 mmol/l, and sodium of 153 mmol/l). He was given a liter of normal saline in the 1st h and another liter in the next 2 h followed by a maintenance fluid rate of 250 ml/h. Three hours after a presentation, he developed generalized seizures and had cardiac arrest twice requiring cardiorespiratory resuscitation. The patient was intubated and subsequently transferred to our hospital.

On arrival at the Intensive Care Unit (ICU), he had bilateral dilated nonreactive pupils. Magnetic resonance imaging (MRI) showed evidence of severe CE with widespread infarcts in the watershed areas with minimal subarachnoid hemorrhage [Figure 1]. Due to his late referral, he was treated conservatively, and no mannitol was given. On the 7th day after admission, mannitol was given. Four days later, he started to show improvement and was extubated. During the next 2 weeks, he showed progressive improvement in his intellectual and motor function. Eventually, he was discharged in good condition with little memory impairment.

### Case 2

A 17-year-old girl presented to the emergency room with severe DKA in a coma. Her initial laboratory workup revealed a pH of 6.79, bicarbonate of 2mmol/l, and serum glucose of

| Access this article online | |
|---|---|
| Quick Response Code: | Website: www.jnsmonline.org |
| | DOI: 10.4103/JNSM.JNSM_15_18 |

**Address for correspondence:** Dr. Riad A. Sulimani, Department of Medicine, College of Medicine, King Saud University, Riyadh 11323, Saudi Arabia.
E-mail: rsulimani@ksu.edu.sa



**How to cite this article:** Sulimani RA, Jammah AA, Ghozzi IM, Alotair HA, Al-Mohaya SA, Ashour TE. Cerebral injury in diabetic ketoacidosis: Is there a room for conservative management?. J Nat Sci Med 2018;1:82-4.

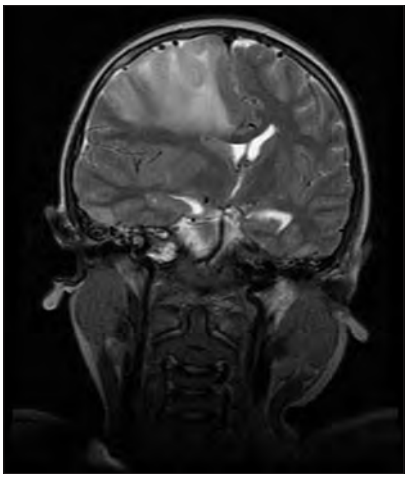

**Figure 1:** Case 1 severe cerebral edema

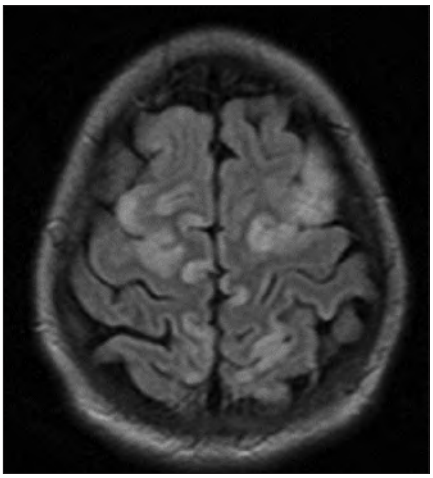

**Figure 2:** Case 2 multiple ischemic areas

43.6mmol/l. MRI showed multiple acute ischemic foci and in the head of left caudate nucleus [Figure 2].

The patient was treated for DKA along the usual measures, including 100 mmol/l of bicarbonate. Aspirin (81 mg) was given through a nasogastric tube. No therapeutic anticoagulant or antithrombolytic therapy was given. She stayed comatose for 24 h. Subsequently, she regained consciousness with evidence of left-sided hemiplegia with right-sided parasethesia. Ten days after, she showed progressive improvement in the motor function on the right side and was able to walk in 3 weeks after discharge with mild difficulty.

## Discussion

The two cases presented were major cerebral insults secondary to severe DKA. The outcome for both was favorable despite initial catastrophic presentations and lack of recommended treatment such as the early use of mannitol in the first case and possible antithrombolytic therapy in the second case.

The first case is a classic severe case of CE with multiple ischemic areas causing two cardiac arrests in which mannitol were not used initially. The second case displays a pattern of diffuse ischemia and multiple infarcts with reversible hemiplegia.

CE, in the first case, was diagnosed on clinical and radiological evidence. In the second case, the presentation was that of ischemic brain injury but with no clinical or radiological findings suggestive of CE.

In general, the pathogenesis of brain insult in DKA is not well understood. Three proposed mechanisms were suggested: vasogenic edema,[7] osmotic edema from aggressive fluid therapy,[8] and ischemia with cytotoxic edema.[9] It is likely that individual pathologic mechanism, or all three, could be operating to different degrees in the affected patient. Use of overzealous fluid replacement therapy, especially in the first few hours of therapy (as in our first patient) and early use of insulin therapy could be blamed for the resulting osmotic edema in this patient.[10] The issue of careful rehydration, particularly in children with severe DKA, prolonged hyperglycemia and severe dehydration has been emphasized in previous guidelines and reviews.[11,12] When the patient is aggressively rehydrated, water will flow into the brain excessively with resultant increased intracranial pressure.[13] Treatment with an early large bolus of insulin could aggravate the situation by metabolizing glucose to water, could further aggravate the situation.[13]

The issue for the use of mannitol in the management of CE in DKA, which was not administered immediately in this patient due to his late arrival to our hospital has been well emphasized.[14,15] However, in a report by Marcin *et al*., it was revealed that the use of mannitol was not particularly associated with better outcome, neither was it associated with a worse effect. It was suggested in this study that the majority of patients had severe CE which may not be applicable to many other patients with milder degrees of CE.[16] The second case displays a pattern of diffuse ischemia and multiple such as cerebral infarcts but with no CE. A decrease in N-acetylaspartate (which is a marker of neuronal integrity) was documented, especially in areas such as basal ganglia, occipital, and gray matter in these patients.[9]

It has been emphasized that DKA is an inflammatory condition characterized by elevated levels of inflammatory markers caused by oxidative stress accompanied by diffuse endothelial injury.[17] Such a proinflammatory state may be chronic in Type 1 diabetic patients,[18] and it gets exaggerated under the oxidative stress caused by hyperglycemia and ketosis. In addition, there is evidence of a procoagulant state in these patients, and such coagulation defects are multiple including low protein C, low protein S, elevated factor VIII and factor V, and changes and increased platelet aggregation.[19]

In our patient, only aspirin and prophylactic subcutaneous heparin were given. Although guidelines for management of stroke in young patients are not well established, the use of thrombolytic therapy has been well documented in case

reports with favorable outcome.[20] Otherwise, use of alteplase (tPA) is not approved in the United States Food and Drug Administration for use in children <18 years of age. Until evidence-based guidelines for the use of thrombolytic therapy are available, we suggest that general good care along with aspirin may be sufficient in some of these patients. In addition, our patient did not receive full therapeutic anticoagulant therapy – whether acutely or for a long-term.

## Conclusion

Select patients with DKA presenting with brain injury may have a good outcome from conservative management. In the case of CE, adherence to the available guidelines in terms of mannitol use is still necessary if the patient is managed within the proper time window. However, good recovery may still be achieved with good ICU care while there is still room for mannitol to be given during the subsequent course of illness if deemed necessary. By the same token, DKA associated cerebral ischemic event may be successfully managed with aspirin with no need for thrombolytic treatment and in the absence of a definite indication for anticoagulation, for example, atrial fibrillation.

### Financial support and sponsorship

Nil.

### Conflicts of interest

There are no conflicts of interest.

## References

1. Glaser N, Barnett P, McCaslin I, Nelson D, Trainor J, Louie J, *et al.* Risk factors for cerebral edema in children with diabetic ketoacidosis. The pediatric emergency medicine collaborative research committee of the American academy of pediatrics. N Engl J Med 2001;344:264-9.
2. Edge JA, Hawkins MM, Winter DL, Dunger DB. The risk and outcome of cerebral oedema developing during diabetic ketoacidosis. Arch Dis Child 2001;85:16-22.
3. Wootton-Gorges SL, Glaser NS. Imaging of the brain in children with type I diabetes mellitus. Pediatr Radiol 2007;37:863-9.
4. Foster JR, Morrison G, Fraser DD. Diabetic ketoacidosis-associated stroke in children and youth. Stroke Res Treat 2011;2011:219706.
5. Wolfsdorf J, Craig ME, Daneman D, Dunger D, Edge J, Lee W, *et al.* Diabetic ketoacidosis in children and adolescents with diabetes. Pediatr Diabetes 2009;10 Suppl 12:118-33.
6. Eleftheriou D, Ganesan V. Controversies in childhood arterial ischemic stroke and cerebral venous sinus thrombosis. Expert Rev Cardiovasc Ther 2009;7:853-61.
7. Jeha George S, Haymond MW. Cerebral edema in children with diabetic ketoacidosis. In: Wolfsdorf JI, Hoppin AG, editors. Up to Date Endocrinology. Waltham, MA: Up To Date; 2011.
8. Silver SM, Clark EC, Schroeder BM, Sterns RH. Pathogenesis of cerebral edema after treatment of diabetic ketoacidosis. Kidney Int 1997;51:1237-44.
9. Wootton-Gorges SL, Buonocore MH, Kuppermann N, Marcin J, Dicarlo J, Neely EK, *et al.* Detection of cerebral {beta}-hydroxy butyrate, acetoacetate, and lactate on proton MR spectroscopy in children with diabetic ketoacidosis. AJNR Am J Neuroradiol 2005;26:1286-91.
10. Edge JA, Jakes RW, Roy Y, Hawkins M, Winter D, Ford-Adams ME, *et al.* The UK case-control study of cerebral oedema complicating diabetic ketoacidosis in children. Diabetologia 2006;49:2002-9.
11. Fiordalisi IG, Harris RM. Pediatric DKA: fluids and Insulin, How Much and When? Practical Summaries in Acute Care. Atlanta: Perkin; 2008.
12. Rosenbloom AL. Cerebral edema in diabetic ketoacidosis. J Clin Endocrinol Metab 2000;85:507-9.
13. Finberg L. Appropriate therapy can prevent cerebral swelling in diabetic ketoacidosis. J Clin Endocrinol Metab 2000;86:508-9.
14. Muir AB, Quisling RG, Yang MC, Rosenbloom AL. Cerebral edema in childhood diabetic ketoacidosis: Natural history, radiographic findings, and early identification. Diabetes Care 2004;27:1541-6.
15. Dunger DB, Sperling MA, Acerini CL, Bohn DJ, Daneman D, Danne TP, *et al.* European society for paediatric endocrinology/Lawson wilkins pediatric endocrine society consensus statement on diabetic ketoacidosis in children and adolescents. Pediatrics 2004;113:e133-40.
16. Marcin JP, Glaser N, Barnett P, McCaslin I, Nelson D, Trainor J, *et al.* Factors associated with adverse outcomes in children with diabetic ketoacidosis-related cerebral edema. J Pediatr 2002;141:793-7.
17. Romano M, Pomilio M, Vigneri S, Falco A, Chiesa PL, Chiarelli F, *et al.* Endothelial perturbation in children and adolescents with type 1 diabetes: Association with markers of the inflammatory reaction. Diabetes Care 2001;24:1674-8.
18. Doğruel N, Kirel B, Akgün Y, Us T. Serum soluble endothelial-cell specific adhesion molecules in children with insulin-dependent diabetes mellitus. J Pediatr Endocrinol Metab 2001;14:287-93.
19. Carr ME. Diabetes mellitus: A hypercoagulable state. J Diabetes Complications 2001;15:44-54.
20. Benedict SL, Ni OK, Schloesser P, White KS, Bale JF Jr. Intra-arterial thrombolysis in a 2-year-old with cardioembolic stroke. J Child Neurol 2007;22:225-7.

# A Snoring Man with an Abnormal Sexual Behavior during Sleep

**Aljohara S. Almeneessier[1,2], Ahmed S. Bahammam[2]**

[1]Department of Family Medicine, College of Medicine, King Saud University, [2]Department of Medicine, The University Sleep Disorders Center, College of Medicine, King Saud University, Riyadh, Saudi Arabia

## Case Report

A 38-year-old male presented with complaints of loud snoring at night for 5 years. His wife noticed that he stops breathing during sleep for a few seconds followed by a big snort. He also complained of choking attacks during sleep, dry mouth, and headache on awakening and unrefreshing sleep. He denied excessive daytime sleepiness but complained of daytime fatigue and decreased concentration.

On further questions, he reluctantly mentioned that he had had sexual relations during sleep with his wife three nights per week for 3 years. On several occasions, these sexual relations take a violent nature usually occurring in the first half of the night. In addition, his wife told him that he used to say sexual terms and phrases during sleep that are not appropriate. He had no recollection of this in the morning. He was surprised when his wife told him about these recurrent actions and started to have feelings of guilt and shame. In addition, the patient mentioned that he started to have marital problems, as his wife thinks that a genie (Jinn) is controlling his body and soul, and became scared to sleep with him in the same bed. The patient has no history of other medical illnesses; however, he admitted to having a history of sleepwalking during childhood and a family history of sleepwalking. There was no history of regular use of medications or alcohol.

Physical examination revealed a body mass index 28.6 kg/m$^2$, neck girth 16 inches, and a Mallampati class of II designation. The remaining physical examination was normal.

Figures 1 and 2 show epochs of the overnight sleep study of the patient.

Overnight polysomnography revealed frequent obstructive respiratory events during sleep [Figure 1] with an apnea–hypopnea index of 37/h. In addition, the patient had multiple similar spells of arousals occurred exclusively from nonrapid eye movement sleep (NREM) sleep (particularly slow wave sleep) that were associated with movements in the bed [Figure 2]. No epileptiform activity was recorded on the limited electroencephalogram recording during these episodes.

The patient was diagnosed to have obstructive sleep apnea (OSA) and was started on continuous positive airway pressure therapy at home during sleep. The patient returned to the clinic for follow-up after 3 weeks and stated that the abnormal sleep behaviors have disappeared.

## What is the Diagnosis?

### Discussion

Figure 1 shows a zoomed 2-min epoch showing obstructive apneas. Airflow is absent despite persisting respiratory paradoxical effort. The obstructive events are followed by arousal and desaturation. The sleep behavior that occurs during sleep in this patient is a type of parasomnia. Parasomnias ("para" means next to, and "somnus" means sleep) comprise a group of the most challenging, fascinating, and unusual behavioral disorders that occur during sleep, which are characterized by sleep-related acute, abnormal behavioral or physiological events. The International Classification of Sleep Disorders, Third Edition (ICSD-3) defines parasomnias as undesirable physical events or experiences that occur during entry into sleep, within sleep, or during arousal from sleep.[1] Human physiological consciousness states consist of wake, NREM sleep, and REM sleep. Parasomnias may occur during NREM, REM, or during transitions to and from sleep.[1] It is thought that parasomnias are associated with central nervous system activation, increases in skeletal muscle activity, and autonomic nervous system changes.[2] Parasomnias may result in sleep disruption and physical harm to affected individuals or the bed partner. NREM parasomnias tend to run in families; it

**Address for correspondence:** Dr. Ahmed S. Bahammam, Department of Medicine, University Sleep Disorders Center, College of Medicine, King Saud University, Riyadh, Saudi Arabia.
E-mail: ashammam2@gmail.com

**Access this article online**

Quick Response Code:

Website: www.jnsmonline.org

DOI: 10.4103/JNSM.JNSM_36_18



**How to cite this article:** Almeneessier AS, Bahammam AS. A snoring man with an abnormal sexual behavior during sleep. J Nat Sci Med 2018;1:85-8.

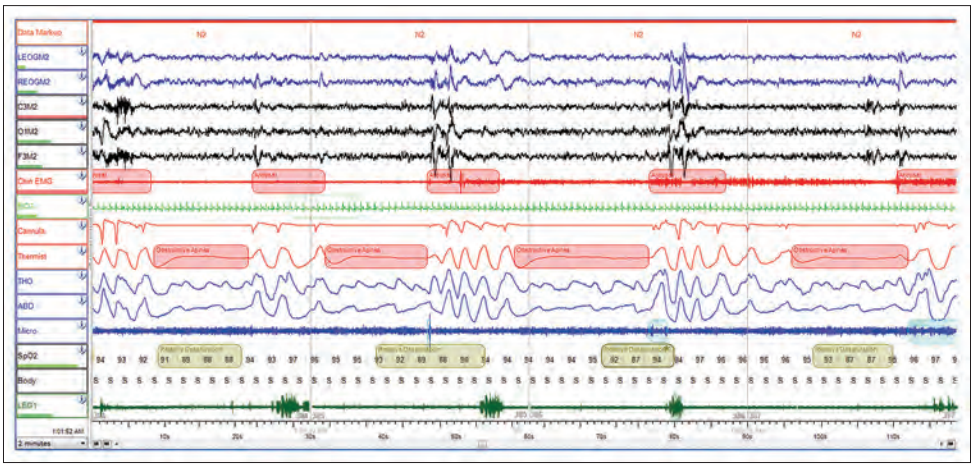

**Figure 1:** A zoomed 2-min epoch showing obstructive apneas. Airflow is absent despite persisting respiratory paradoxical effort. The obstructive events are followed by arousal and desaturation and snorting sounds

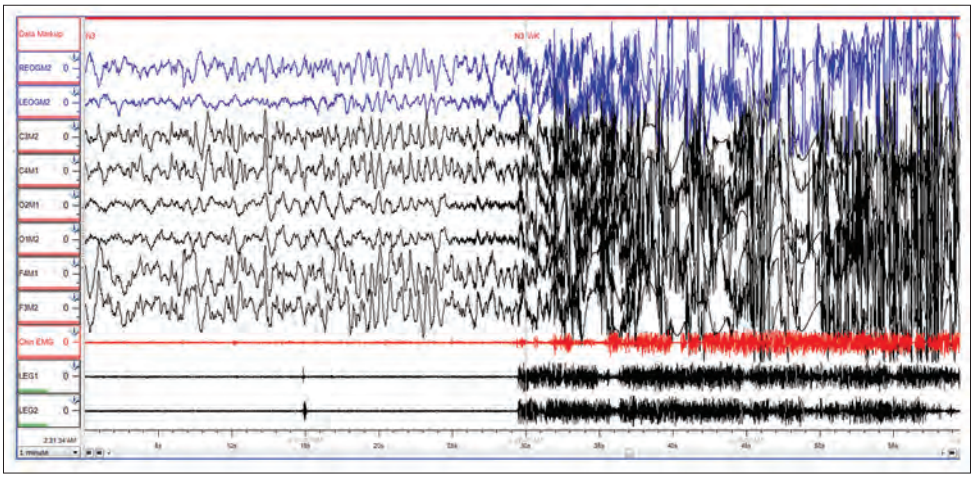

**Figure 2:** A 30-s epoch of the sleep study; in the first half, the patient was in stage N3 (slow wave nonrapid eye movement sleep), and in the second half of the epoch, there is an increase in chin and legs electromyographic tone indicating movement

has long been suspected that genetic factors are involved.[2] In general, patients with parasomnias receive wrong diagnoses, and the correct diagnosis is usually delayed.[3]

The ICSD-3 classifies NREM parasomnias into confusional arousal, sleep terror, sleepwalking and sleep-related eating disorder; and REM parasomnias into REM sleep behavior disorder, recurrent isolated sleep paralysis, and nightmare disorder.[1] NREM parasomnias are usually observed early in the sleep period when the slow wave sleep pressure is most pronounced as occurred in the present case.

The discussed patient has a parasomnia called "sexsomnia," which is considered to be an NREM parasomnia. Figure 2 shows a 30-s epoch of the sleep study; in the first half of the epoch, the patient was in stage N3 (slow-wave NREM sleep), and in the second half of the epoch, there is an increase in chin and legs electromyographic tone indicating movement.

Sexsomnia was described in 2003, and is considered as a clinical variant of confusional arousal or sleepwalking depending on the observed behavior.[1,4] So far, approximately, 95 published cases have been described; therefore, clinical descriptions of sexsomnia are based on case reports and small series.[5] Sexsomnia in our patient is triggered by OSA-induced arousals, as different parasomnias have been reported to be triggered by sleep deprivation, alcohol, some medications such as serotonin-reuptake inhibitors (SSRIs), and other sleep disorders such as periodic leg movements or endogenous triggers such as pain or a full bladder.[6] Therefore, a detailed medication history with the bed partner's assistance is essential in the evaluation of a patient with sexsomnia.

The characteristic features of sexsomnia include sexual arousal accompanied by autonomic activation such as nocturnal penile tumescence, vaginal lubrication, nocturnal emission, and dream orgasm.[7] In a series of 49 patients with sexsomnia, males represented 75%, with a mean age of onset of 28 years and mean age at presentation of 35 years.[8] A wide-range of sexual behaviors had been reported including sexual intercourse/attempted intercourse (49%),

fondling the bed partner (40%), agitated/assaultive sexual behaviors (37%), masturbation (23%), sexsomnia with minors (20%), sexual vocalizations (19%), and spontaneous sleep orgasm (4%).[8] The abnormal sexual behavior can affect any person co-sleeping with the patient in the same room. Therefore, it is not surprising that sexsomnia may lead to adverse legal consequences.[8,9]

The exact prevalence of abnormal sexual behavior during sleep is unknown. However, insights into the prevalence of sleep-related abnormal sexual behaviors (suggestive of sexsomnia) have been reported in a retrospective analysis of medical records of sleep medicine patients to be 7.6%.[10] An epidemiologic study from Norway that estimated lifetime and current prevalence of various parasomnias in the general population using a telephone interview revealed that lifetime and current prevalence of sexual acts during sleep were 7.1% and 2.7%, respectively.[11] Nevertheless, the authors stressed that the results need to be interpreted with caution due to methodological shortcomings, such as a low response rate to participate in the telephone interview, and the single questions used in the survey.[11]

In the present case, the patient did not present the abnormal sexual behavior during sleep as the primary complaint. In fact, he volunteered the information after questioning about abnormal sleep behaviors. This probably, reflects the sensitive nature of the complaint and the associated feelings of guilt and shame, which underscore the need to ask patients, and their bed partners, about comorbid parasomnias when evaluating patients with OSA symptoms.

General practitioners, neurologists, and psychiatrists should be aware of sexsomnia presentation and diagnosis as they are often consulted for evaluation of unusual behaviors, including sleep-related abnormal sexual behaviors. However, it is important to realize that sleep-related sexual behaviors may rarely arise from nocturnal seizures, which can often be successfully treated.[12] A striking contrast between ictal sexsomnia episodes and sexsomnia is the fact that following an ictal sexsomnia episode, the patient usually has a high rate of recall for the episode, while there is amnesia after sexsomnia.[8] Nevertheless, differentiating nocturnal seizures from parasomnia can occasionally be difficult on clinical grounds alone; therefore, a multidisciplinary approach is required in some cases.

Current data on the treatment of sexsomnia are limited. The therapeutic approach should include both behavioral and pharmacological treatments. The patient should know that sleep deprivation and exhaustion before sleep may provoke the occurrence of parasomnia, as increased sleep debt results in rebound slow-wave sleep. In addition, avoidance of alcohol and medications that are known to trigger parasomnias is essential. If a comorbid sleep disorder that is known to trigger parasomnias is discovered, it should be treated before initiating pharmacological treatment. Treatment of the comorbid sleep disorder may result in complete disappearance of the parasomnia and alleviate the need for pharmacological treatment as occurred in the present case. In one series, 86% of patients had control of sexsomnia with bedtime clonazepam.[8] Clonazepam at a dose of 0.5–1 mg at night is effective in most cases. Although SSRIs have been shown to provoke sexsomnia in some patients, recently, paroxetine 5–10 mg in the evening have been reported to be effective in some patients.[5]

### Final diagnosis

Sexsomnia triggered by OSA with complete elimination of sexsomnia after treating OSA.

## Clinicopathological Pearls

1. Sexsomnia is classified as a subtype of NREM parasomnia disorders of arousal in the ICSD-3
2. Amnesia of the episode is an important feature of all NREM parasomnias, including sexsomnia
3. There is no evidence that sexsomnia is secondary to psychiatric conditions, or is the result of sexual frustration/repression
4. Treatment of comorbid sleep disorders such as OSA may result in a complete cure of the parasomnia.

### Declaration of patient consent

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

### Financial support and sponsorship

This work was supported by the Strategic Technologies Program of the National Plan for Sciences and Technology and Innovation in the Kingdom of Saudi Arabia (MED511-02-08). The study sponsors played no role in the study design, the collection, analysis or interpretation of data, writing the manuscript, or the decision to submit the manuscript.

### Conflicts of interest

There are no conflicts of interest.

## References

1. American Academy of Sleep Medicine. International Classification of Sleep Disorders (ICSD). 3rd ed. Darien, IL: American Academy of Sleep Medicine; 2014.
2. Seithikurippu Ratnas PP, BaHammam AS, Shapiro CM. Parasomnias. In: Stolerman IP, Price LH, (eds). Encyclopedia of Psychopharmacology. Springer, Berlin, Heidelberg; 2015. p. 1210-8.
3. Avidan AY, Kaplish N. The parasomnias: Epidemiology, clinical features, and diagnostic approach. Clin Chest Med 2010;31:353-70.
4. Shapiro CM, Trajanovic NN, Fedoroff JP. Sexsomnia – A new parasomnia? Can J Psychiatry 2003;48:311-7.
5. Dubessy AL, Leu-Semenescu S, Attali V, Maranci JB, Arnulf I. Sexsomnia: A Specialized non-REM parasomnia? Sleep 2017;40. doi: 10.1093/sleep/zsw043. [Epub ahead of print].
6. Guilleminault C, Palombini L, Pelayo R, Chervin RD. Sleepwalking and sleep terrors in prepubertal children: What triggers them? Pediatrics 2003;111:e17-25.
7. Andersen ML, Poyares D, Alves RS, Skomro R, Tufik S. Sexsomnia: Abnormal sexual behavior during sleep. Brain Res Rev 2007;56:271-82.

8. Schenck CH. Update on sexsomnia, sleep related sexual seizures, and forensic implications. NeuroQuantol 2015;13:518-41.
9. Irfan M, Schenck CH, Howell MJ. Non-rapid eye movement sleep and overlap parasomnias. Continuum (Minneap Minn) 2017;23:1035-50.
10. Chung SA, Yegneswaran B, Natarajan A, Trajanovic N, Shapiro CM. Frequency of sexomnia in sleep clinic patients. Sleep 2010;33:A226.
11. Bjorvatn B, Grønli J, Pallesen S. Prevalence of different parasomnias in the general population. Sleep Med 2010;11:1031-4.
12. Pelin Z, Yazla E. Abnormal sexual behavior during sleep in temporal lobe epilepsy: A case report. Balkan Med J 2012;29:211-3.

Editor-in-Chief Prof. Ahmed BaHammam. Printed and published by Wolters Kluwer India Private Limited on behalf of the College of Medicine, King Saud University, and printed at Dhote Offset Technokrafts Pvt. Ltd. Goregaon (E), Mumbai, India and published at A-202, 2nd Floor, The Qube, Andheri (East), Mumbai, India.