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Editorial Office
Room # 126, 1st Floor,
Doctors' Plaza, Opp.
Ayub Medical College,
Abbottabad-22040,
Pakistan.
Cell: +92-314-**5000900**
Email: pjp@pps.org.pk
URL: www.pjp.pps.org.pk

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EDITORIAL

LIMITATIONS OF HUMAN SENSORY SYSTEMS

Tehseen Iqbal
RYK Medical College, Rahim Yar Khan, Pakistan

All living organisms on earth have sensory systems which help them to detect changes in their environment so that they can respond appropriately to survive, protect themselves or grow. While our senses are incredible tools for perceiving and interacting with the world, they have limitations. Our eyes can only detect a narrow range of the electromagnetic spectrum, we have narrow field of vision and limited visual acuity. Our ears have a limited range of frequencies that they can detect, preventing us from hearing infrasound and ultrasound. We have relatively small number of olfactory receptors. Humans have around 9,000 taste buds, allowing us to detect a wide range of flavours. In contrast, cats have only around 470 taste buds, making them less sensitive to taste. Dogs, on the other hand, have around 1,700 taste buds but their sense of smell is more dominant in determining their food preferences. By understanding these limitations, and knowing about the different mechanisms for enhanced or alternative perception present in other organisms, we can develop technologies and methods to compensate for them and enhance our understanding of the world around us. Inspired by the eye of the morpho butterfly, a surgical camera is developed that connects to the goggles of a surgeon who sees infrared signals given off by tumour-binding dyes and surgeon can remove all of the cancerous tissue.

Keywords: Sensory systems, animal senses, non-human senses

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All living organisms on earth have sensory systems which help them to detect changes in their environment so that they can respond appropriately to survive, protect themselves or grow. Living organisms on earth are microbes, plants and animals. Microbes, including bacteria and fungi, have evolved various mechanisms to sense and respond to potential threats in their environment. These mechanisms allow them to detect changes in external conditions and develop adaptive responses to survive and thrive.[1] Additionally, microbes can communicate with each other through a process called quorum sensing.[2] Plants have sophisticated sensory systems to detect and respond to light, gravity, temperature, and physical touch. Receptors sense environmental factors and relay the information to effector systems, often through intermediate chemical messengers, to bring about plant responses.[3] Touch me not plant responds to touch and other stimulation by rapidly closing its leaves and drooping.

We discuss here what senses are more powerful in other animals and what senses other animals have in addition to those present in humans. The normal aging process causes gradual losses to the sensory systems. Generally, these changes begin around the age of 50 years. Fortunately, once limitations are recognized and accepted, adjustments or adaptations to the person's environment can help make up for the losses.[4]

Sense of Vision: Our eyes can only detect a narrow range of the electromagnetic spectrum, known as the visible spectrum. We are unable to perceive ultraviolet or infrared light without the aid of technology, although some animals can 'see' with the help of infra-red-light detection. For example, Pit-viper snakes see through two visual mechanisms, i.e., eyes and IR detection mechanism of their pits.[5] Some other animals can also sense the infra-red light, e.g., wolves, dogs, snakes, mosquitos, insects, Vampire bats and bullfrogs. Bullfrogs' eyes adapt to analyze either visible light or infrared light.[6] UV sensitivity is widespread in the animal kingdom. Radiation above 320 and below 400 nm (UVB) can be perceived by many animal species.[7]

Our eyes have a relatively narrow field of vision, with only a small area in the centre called the fovea providing high visual acuity. This means that we often miss important details in our peripheral vision. Horses' eyes are located on the side of their head, so they have a wide range of vision. They can see almost 360 degrees.[8] Owls have a wide visual span of up to 110 degrees, enabling them to spot prey from various angles. Their ability to rotate their heads up to 270 degrees further enhances their visual field.[9]

Another limitation is our visual acuity which refers to the sharpness and clarity of our vision. Humans have excellent visual acuity compared to many other species but some species have visual acuity better than humans. The animal with the sharpest vision is generally agreed to be the peregrine falcon. Scientists estimate that falcon vision is eight times better than humans.[10]

Sense of Hearing: Our ears have a limited range of frequencies that they can detect (20 to 20,000 Hz), preventing us from perceiving sounds below or above a certain frequency. Animals that communicate using infrasonic and ultrasonic sounds are bats, dolphins, dogs, frogs, toads, etc. which communicate via ultrasonic sounds. Rhinos, hippos, elephants, whales, octopuses, pigeons, squid, cuttlefish, cod, Guinea fowl, etc. communicate via infrasonic sounds.[11]

Sense of Smell: Like any other sensory system, the

human sense of smell has its limitations. One limitation is the relatively small number of olfactory receptors humans possess compared to other animals. Humans have around 400 different types of olfactory receptors. The bloodhound is considered to have the sharpest sense of smell among animals. They possess approximately 300 million scent receptors, which is about 40 times more than humans. According to a study published in the journal 'Comparative Biochemistry and Physiology' in 2004, bloodhounds possess more olfactory receptor genes than any other known species.[12]

Sense of Taste: The human sense of taste is a complex and fascinating mechanism that allows us to perceive and enjoy the flavours of various foods and beverages. The sense of taste varies greatly among different animal species. For instance, humans have around 9,000 taste buds, allowing us to detect a wide range of flavours. In contrast, cats have only around 470 taste buds, making them less sensitive to taste. Dogs, on the other hand, have around 1,700 taste buds, but their sense of smell is more dominant in determining their food preferences. Interestingly, some animals, like sharks, have taste buds not only in their mouths but also on their skin, enhancing their ability to detect prey.[13]

Some animals can detect forms of energy invisible to us, like magnetic and electrical fields. We cannot sense the faint electric fields that sharks and platypuses can. We are not privy to the magnetic fields that migrating birds and sea turtles detect. We can't trace the invisible trail of a swimming fish the way a seal can. We can't feel the air currents created by a buzzing fly the way a wandering spider does. Our ears cannot hear the ultrasonic calls of rodents and hummingbirds or the infrasonic calls of elephants and whales. Moths can hear the ultrasonic calls of echolocating bats. Our eyes cannot see the infrared radiation that rattlesnakes detect. Our eyes cannot see the ultraviolet light that the birds and the bees can sense. Arctic reindeer can see ultraviolet light. Many birds like the zebra finch, can see extra colours, their retinas possess four different types of colour-sensing cones. Mosquitos follow the scent of exhaled carbon dioxide.[14,15]

In conclusion, while our senses are incredible tools for perceiving and interacting with the world, they have limitations. By understanding these limitations, and knowing about the different mechanisms for enhanced or alternative perception present in other organisms, we can develop technologies and methods to compensate for them and enhance our understanding of the world around us. Researchers at the University of Illinois and Washington University have developed a surgical camera inspired by the eye of the morpho butterfly. The camera, connected to the goggles a surgeon wears, sees infrared signals given off by tumour-binding dyes so that the surgeon can remove all of the cancerous tissue.[16] Electroreception, magnetoreception, infrared perception, ultraviolet vision and echolocation are some of the senses which are present in some animals but not in humans.

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Address for Correspondence:

Prof Dr Tehseen Iqbal, Head of Physiology Department and Vice Principal, RYK Medical College, Rahim Yar Khan, Pakistan. **Cell:** +92-333-6144799

Email: prof.tehseeniqbal@gmail.com

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ORIGINAL ARTICLE

EFFECT OF WHITE SUGAR, BROWN SUGAR AND JAGGERY POWDER SYRUP ON BIOCHEMICAL AND HISTOPATHOLOGICAL MARKERS OF SPRAGUE-DAWLEY RATS

Hifza Noor Lodhi, Sana Rasheed Chaudhry*, Shagufta Nasreen, Amer Hassan Siddique***, Rana Muhammad Yousaf†, Sadia Chiragh††**

Department of Physiology, Rashid Latif Khan University Medical College, Lahore, *Physiology, **Anatomy, ***Pharmacology, Postgraduate Medical Institute/Ameer-ud-Din Medical College/Lahore General Hospital, Lahore, †Central Research Laboratory, Lahore General Hospital, Lahore, ††Department of Pharmacology, Al-Aleem Medical College, Lahore, Pakistan

Background: There is a common myth that white sugar produces more harm as compared to brown sugar and jaggery powder which may contain useful chemicals other than sucrose. This study was aimed to assess this common belief by evaluating different parameters in Sprague dawley rats. **Methods:** This 12-weeks study was carried out at Postgraduate Medical Institute, Lahore from Sep to Nov 2018. Sucrose content of white sugar, brown sugar and jaggery powder was determined and a 10% sucrose syrup was given to twenty male adult Sprague dawley rats divided into four groups. Group A: normal control (plain drinking water), group B: white sugar group (10% sucrose syrup), group C: brown sugar group and group D: jaggery powder group for 12 consecutive weeks. Body-weight, non-invasive blood pressure and biochemical tests were performed. Liver, pancreas and kidney sections prepared and examined under light microscope. **Results:** No significant differences were observed between body-weight, blood-pressure, serum insulin, lipid profile, and renal function tests. Blood sugar was higher in all experimental groups than normal control with no statistical differences in between. Liver enzymes especially AST and ALP were significantly deranged than normal control in brown sugar group. Liver weight was also significantly higher in brown and white sugar groups. Histology of liver, pancreas and kidney sections showed congestion and inflammatory changes in all experimental groups. **Conclusion:** Consecutive administration of all three types of sugar produced no significant differences except for liver weight and enzymes which were the most deranged in brown sugar group.

Keywords: Sugar, Jaggery powder, Histopathology, Liver, Pancreas, Kidney, Marker, Sprague-dawley

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INTRODUCTION

Hypertension remains a major risk factor for stroke, cardiovascular disease, renal disease, and death. It accounts for 10% of the total annual health budget in developed countries.[1] By 2025, the number of people living with hypertension is expected to reach 1.56 billion people.[2]

Dietary factors that increase blood pressure (BP) are of interest to public health authorities.[3,4]

The introduction of refined sugars into the food supply and the subsequent rise in sugar consumption has mirrored the increase in the prevalence of hypertension over the last century.[3]

Sucrose is the most frequently used, and admired component to obtain sweetness in human food preparation. It is a disaccharide containing two monosacharides fructose and glucose chemically linked together. Sucrose is used in various products as industrial sweetener such as drinks, confectionary, jams, jellies and preserves. It is primarily esteemed for its sweetness and serves as a vital source of energy, yielding 394 kcal/100 g of refined sugar.[5]

Increases in ingestion of sucrose have shown to elevate the blood pressure in experimental animals.[6] Furthermore, reduced consumption of sugar-sweetened beverages, as well as sugars in the form of sucrose, glucose and fructose, was found to be significantly associated with reduced BP in a prospective study.[7]

The mechanism by which sucrose increases the blood is by causing the stimulation of the sympathetic nervous system thus leading to an increase in Renin secretion which leads to renal sodium retention and vascular resistance. Other probable mechanism involved in the sucrose induce hypertension can be insulin resistance and hyperinsulinemia which has been shown to develop when normal rats eat a high sucrose diet.[8]

Added sugars perhaps matter more than dietary sodium for hypertension. Naturally occurring sugars are not harmful for health however added sugar is a problem and should be targeted more clearly in dietary guidelines to sustain cardio-metabolic and general health.

METHODOLOGY

This 12-weeks experiment was carried out in Postgraduate Medical Institute, Lahore, Pakistan from Sep to Nov 2018. Ethical approval was granted by the

Institutional Review Committee. Guidelines laid in Helsinki declaration by WMA in 2013 for animal research were followed strictly.[1]

Twenty adult male Sprague-dawley rats that weighed 150–200 grams were first acclimatized to the environment of the animal house for one week. They were kept at standard temperature (22±3 °C) and under 12 hours light-and-dark cycles. Standard rat chow was administered *ad libitum* to all rats.

Rats were then randomly divided into four groups ($n=5$) using lottery method. A 10% sucrose syrup was prepared based on sucrose content found in three types of sugars (99% for white sugar, 91% for brown sugar and 75% for jaggery powder) analyzed at Pakistan Council of Scientific and Industrial Research Laboratories, Lahore. Therefore, Group A was given plain drinking water being normal control, group B was given 10.10 grams white sugar dissolved in 100 ml drinking water, group C was given 11.00 grams brown sugar dissolved in 100 ml drinking water, and group D was given 13.33 grams jaggery powder dissolved in 100 ml drinking water for 12 consecutive weeks.

Body weight and blood pressure of all rats was measured for 12 consecutive weeks. An inflatable cuff around rat tail was applied and blood pressure was measured non-invasively using computer-based data recording system PowerLab® model: M-ML 856, Australia. Three reading were taken from each rat per week and an average reading was calculated for that week.

After the completion of 12 weeks, rats were anaesthetized with chloroform and blood sample was drawn through cardiac puncture. The blood was allowed to clot and then centrifuged at 4,000 rpm for 10 minutes for serum separation. Serum glucose, serum insulin, liver function tests (Serum total bilirubin, ALT, AST and ALP), serum urea and creatinine and lipid profile (triglycerides, total cholesterol, LDL and HDL) were determined using commercially available kits made by CELM diagnosis, São Paulo, Brazil.

All rats were then euthanized, and their liver, kidneys and pancreas were dissected out, weighed and stored in 10% NBF. Tissues were processed and cut into 5 µm thick section placed on glass slides. These slides were stained with haematoxylin and eosin and analysed using 10× and 40× magnifications.

SPSS-25 was used for statistical analysis. Shapiro-Wilk test was used to check normality of the data. The quantitative variables found to be normally distributed were expressed as Mean±SD, while qualitative and non-normally distributed quantitative variables were expressed as median and interquartile ratio. Analysis of variance (ANOVA) and post-hoc Tukey test were used determine differences among and between groups in case of normally distributed data, while Kruskal-Wallis ANOVA and Mann-Whitney U tests were applied to see difference between non-normally distributed variables; and $p\leq0.05$ was considered statistically significant.

RESULTS

The body weight, non-invasive blood pressure and lipid profile of all rats had no statistically significant difference at the end of study (Table-1).

Serum glucose and serum insulin in all experimental groups were non-significantly different at the end of study. It was, however, numerically higher than normal control group in all experimental groups at the end of study (Table-2).

Apparently, brown sugar group had the most deranged liver enzymes. Statistically significant difference was found in ALP and AST of rats. Upon post-hoc Tukey test, brown sugar group had significantly higher ALP ($p=0.037$) than normal control while the difference among experimental groups was non-significant. Similarly, AST was significantly higher in brown sugar group ($p=0.004$) than jaggery powder group. Rest of the tests had non-significant differences (Table-3).

Liver weight was significantly different among all groups. It was highest in white sugar and brown sugar ($p=0.007$ and 0.006) followed by jaggery powder group ($p=0.049$).

Though serum urea was significantly different among all groups, it was numerically highest in normal control group which makes this finding unremarkable. Serum creatinine and uric acid had no statistical differences (Table-4).

Table-1: Body weight, blood pressure, and lipid profile of rats in normal control and experimental groups (n=5) (Mean±SD)

Parameter at 12 weeks	Normal Control	White Sugar	Brown Sugar	Jaggery Powder	*p*
Body weight (Gm)	271.2±9	282±25	270.2±8	293±14	0.673
Blood pressure (mmHg) at week 6	103.9±11	112.8±12	107±6	99.4±9	0.237
Blood pressure (mmHg) at week 12	104.5±6.8	109.8±9.5	112.7±13	103.3±10	0.283
Cholesterol (mg/dL)	78±13	90±12	67.2±16	72±6	0.064
Triglycerides (mg/dL)	61±4	68.6±16	58±4	54.8±9	0.183
HDL (mg/dL)	26±9	31±10	20±3	20±5	0.113
LDL (mg/dL)	39.4±12	45±12	35±16	41±2	0.671

Table-2: Serum glucose and serum insulin of rats in normal control and experimental groups

Parameter at 12 weeks	Normal Control	White Sugar	Brown Sugar	Jaggery Powder	*p*
	Mean±SD				
Serum glucose (mg/dL)	108.8±23	248.4±109	269.8±121	227.60±3	0.078
Serum insulin (mIU/L)	107±53	78±65	67±76	93±31	0.724

Table-3: Liver function tests of rats in normal control and experimental groups (n=5), (Mean±SD)

Parameter at 12 weeks	Normal Control	White Sugar	Brown Sugar	Jaggery Powder	*p*
ALP (mg/dL)	188±42	220±22	260±50*	252±30	0.032
ALT (mg/dL)	48±9.9	48±4.3	123±101	50±5.3	0.084
AST (mg/dL)	193±45	198±67	306.1±31^^	102±2	0.007
Bilirubin (mg/dL)	0.36±0.134	0.36±0.15	0.44±0.11	0.38±0.08	0.707
Liver weight in grams	7.3±0.52	9.5±1.04**	9.56±0.91**	8.9±1.02*	0.004

*p=0.037 as compared to normal control, ^^p=0.004 as compared to jaggery group

Table-4: Serum urea, creatinine and uric acid in normal control and experimental groups (n=5), (Mean±SD)

Parameter at 12 weeks	Normal Control	White Sugar	Brown Sugar	Jaggery Powder	*p*
Urea (mg/dL)	62±6	36.8±4	40±6	40±6	0.000*
Creatinine (mg/dL)	0.7±0.1	0.64±0.11	0.62±0.04	0.58±0.08	0.149
Uric acid (mg/dL)	1.84±0.4	2.08±0.73	3.6±1.24	3.2±1.9	0.094

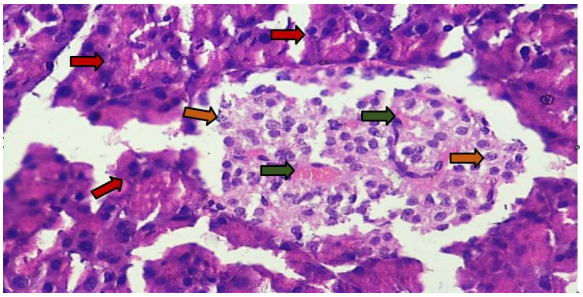

Figure-1a: Pancreatic section of White sugar group. H&E staining 40× magnification

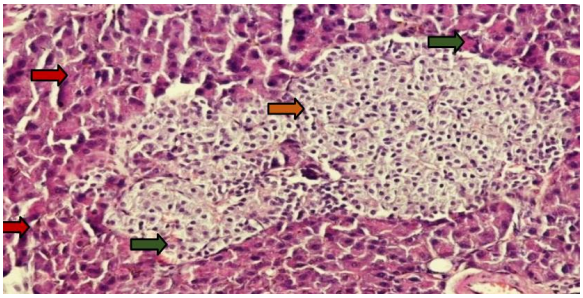

Figure-1b: Pancreatic section of brown sugar group. H&E staining 40× magnification

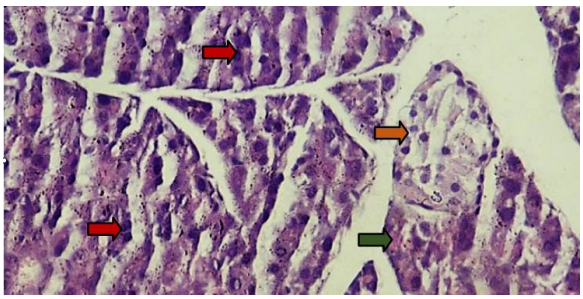

Figure-1c: Pancreatic section of jaggery treated group. H&E staining 40× magnification

Photomicrographs of pancreatic section from all experimental groups showed degenerated cells in islets of Langerhans (brown arrows), congested blood vessels (dark green arrows) and disrupted serous acini (maroon arrows). The changes were the most severe in jaggery powder group and least in brown sugar treated group.

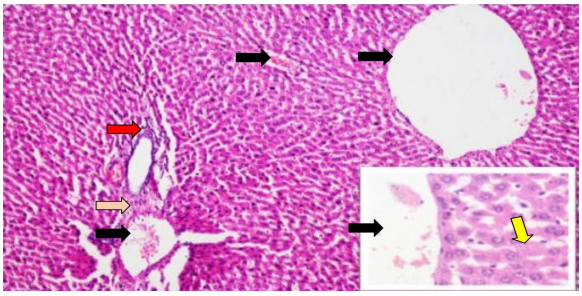

Figure-2a: Liver section of white sugar treated group. H&E staining. 10× and 40× (inbox)

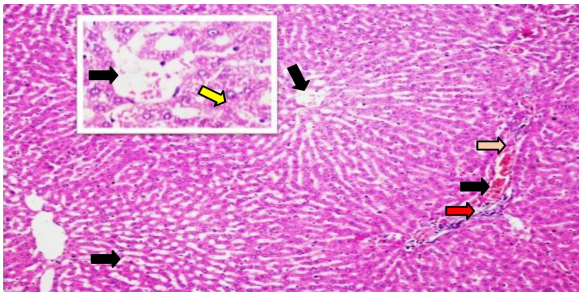

Figure-2b: Liver section of brown sugar treated group. H&E staining. 10× and 40× (inbox)

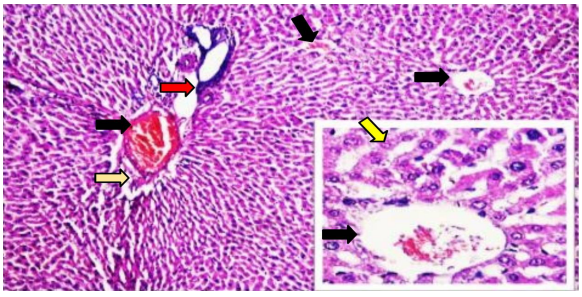

Figure-2c: Liver section of jaggery powder treated group. H&E staining. 10× and 40× (inbox)

Photomicrographs of liver sections under 40x zoom showed central vein, sinusoidal, and portal venous congestion (black arrows), degenerated hepatocytes with vacuolization (yellow arrows), perivenular fibrosis (peach arrows) and inflammatory cell infiltration (red arrows) as seen in Fig 2a, b and c. The changes were most marked in brown sugar group and were the least in white sugar treated group.

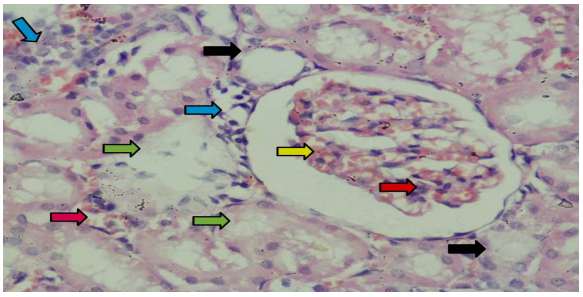

Figure-3a: Renal section in white sugar treated group. H&E staining 40× magnification

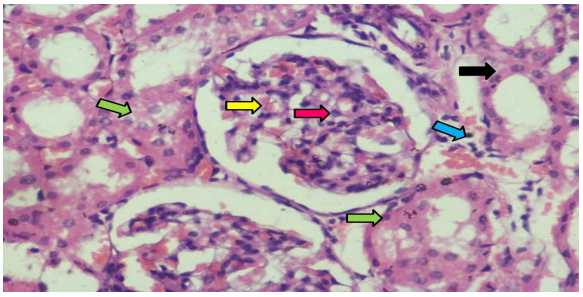

Figure-3b: Renal section in brown sugar treated group. H&E staining. 40× magnification

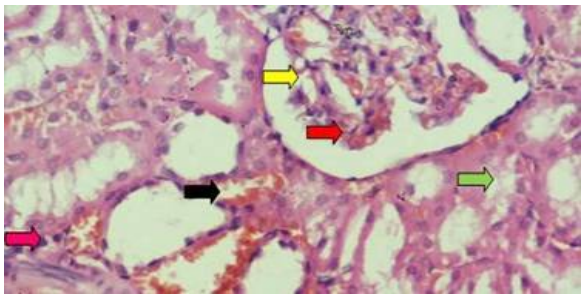

Figure-3c: Renal section in jaggery powder treated group. H&E staining. 40× magnification

Photomicrographs of a kidney sections showed degenerated (Red arrow) & congested glomerulus (Yellow arrow), highly vacuolated proximal (Light green arrow) & distal convoluted (Black arrow) tubules, stromal inflammatory cell infiltrate (Light blue arrow) & blood vessel congestion (Pink arrow). The changes were the most marked in jaggery powder group and the least in brown sugar group.

DISCUSSION

In the present study we aimed to determine the effects of 10% sucrose syrups made from white sugar, brown sugar and jaggery powder on body weight, blood glucose levels, fasting insulin levels, blood pressure, lipid profile and tissue parenchyma of liver, pancreas, and kidneys.

We reported in the present study that the body weight of the rats did increase over time but was not statistically different among the study groups at any point in the study. This finding was consistent with the results reported by a study in which when feed high sucrose 40% diet to rats for 16 weeks.[9]

The fasting blood glucose levels were raised at the end of 12 weeks in the present study but they did not reach statistical significance yet. The same finding was reported by Souza Cruz *et al* when using a higher 40% sucrose diet.[10] Amin *et al* reported that rats given high sucrose diet had significantly larger visceral fat pads and hypertriglyceridemia, however, neither plasma glucose nor insulin levels were significantly higher, while hyperglycaemia and insulin resistance occur after 20 weeks of feeding high sucrose diet.[11]

The weight of the liver in the present study was significantly higher in the rats given sucrose syrup as compared to the control group that is likely due to an increase in liver fat deposits. In the present study we reported a non-significant difference in blood pressure of the rats at end of 12 weeks by using a non-invasive rat tail cuff monitor. This is consistent with findings of a study[9] who recorded blood pressure of sucrose fed rats by insertion of intra-aortic catheter (telemetric method). They reported that there was no statistical difference in the mean 24-hour blood pressure taken on monthly basis for 4 consecutive months. They further added that there was a consistent increase in hourly systolic blood pressure of rats when measured within an hour of providing food. So they suggested that sucrose intake could increase blood pressure during the immediate postprandial period.

In the present study we observed the hepatotoxic effect of jaggery powder and brown sugar evident by a significantly raised serum AST, ALP levels in these groups. The mean Serum ALT levels were highest in brown sugar and jaggery powder syrup groups as compared to the other two groups but difference did not reach statistical significance (p>0,05). The serum fasting cholesterol and triglycerides were not statistically different among the study groups. A study[12] done in 2016 observed that there was no significant difference between serum AST and ALT levels of adult Wister rats given fresh cane brown sugar juice for 4 consecutive weeks while they also reported no significant difference between fasting serum cholesterol and triglycerides which is consistent with the finding of the present study. Another study in 2020 reported that the there was no significant difference in the lipid profile of adult Sprague-dawley rats when given low doses of white and brown sugar but the difference became significant as compared to control when high doses of these two natural sugar sweeteners were given for 12 consecutive weeks.[13]

In the present study the maximum liver parenchymal damage was observed in the brown sugar group when compared to control group. A similar finding was reported by Corona-Pérez *et al* in their study. They reported that the rats given sucrose had morphological alterations in their liver parenchymal cells. The reason being that the sugar ingestion initializes structural changes, including decrease in hepatocyte number and an increased hepatocyte size.

These characteristics are linked to hepatocyte ballooning, which is a common feature of the injured liver.[14] They also reported that there was higher vacuolation in the group given sucrose, which is linked to an increase in cellular deposits and cellular damage.[15] They reported the presence of collagen in the hepatocytes of sucrose-fed animals, and mild fibrosis mainly in the perisinusoidal region and pericentral zone, which relates to Non-Alcoholic Fatty Liver Disease.[16] The higher levels of liver enzymes called transaminases reported in this study also suggest a disruption of the liver function.[17] A study showed comparable effects after 8 weeks of sucrose supplementation, classifying their study results as of moderate-grade fibrosis. In contrast to this, another study[18] found no evidence of liver fibrosis even after giving sucrose supplements for 20 weeks, despite reporting higher lipid content.

In the present study we observed that the maximum histo-pathological changes indicative of pancreatic inflammation were present in the group given jaggery powder syrup when compared to the control group. Same was reported in another study.[9]

CONCLUSION

The rat pancreas and kidneys showed adverse inflammatory histopathological changes maximally in rats taking jaggery powder followed by white sugar. The adverse effects on liver cells were maximally seen on rats given brown sugar. Further work is recommended with larger sample size and different chemical constituents found in jaggery powder and brown sugars so that these adverse changes may be attributed to the proper chemical substance.

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Address for Correspondence:

Dr Hifza Noor Lodhi, Department of Physiology, Rashid Latif Khan University Medical College, Lahore, Pakistan. **Cell:** +92-312-8244496

Email: noor.musa.burki@gmail.com

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Contribution of Authors:

HNL: Concept, study design, data collection, manuscript writing, approval
SN: Data analysis and manuscript writing
RMY: Concept, data collection, final approval
SRC: Data collection, manuscript writing, final approval
AHS: Study design, final approval
SC: Data collection, final approval

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ORIGINAL ARTICLE

ANTI-MÜLLERIAN HORMONE: A BETTER BIO MARKER FOR ASSESSMENT OF ANOVULATION IN POLYCYSTIC OVARIAN SYNDROME COMPARED TO CONVENTIONAL BIOMARKERS

Arfa Goheer, Rabia Saeed*, Iqra Sajid, Hafiza Asma Hafiz, Sara Khan, Ammar Bin Saad*****

Department of Pathology, Quaid-e-Azam Medical College, Bahawalpur, *CMH Institute of Medical Sciences, Bahawalpur, **Shahida Islam Medical and Dental College, Bahawalpur, ***Department of Pathology, Ayub Medical College, Abbottabad, Pakistan

Background: Polycystic Ovary Syndrome (PCOS) is a common endocrine disorder affecting women of childbearing age. The objective of this study was to determine the diagnostic accuracy of Anti-Müllerian Hormone (AMH) in predicting anovulatory PCOS, keeping day 21 progesterone level as gold standard. **Methods:** This cross-sectional validation study was conducted on 289 women aged 15–30 years with PCOS, from 11 Aug 2021 to 10 Feb 2022, in Pathology Department, Bahawal Victoria Hospital, Bahawalpur. AMH levels were measured and anovulatory events documented. AMH levels were compared with progesterone levels on the 21st day of the menstrual cycle. **Results:** In the cohort of individuals demonstrating a positive presence of AMH, a total of 149 were accurately identified as true positives, while 6 were mistakenly classified as false positives. Within the group of patients exhibiting a negative status for AMH, 8 were incorrectly categorized as false negatives, whereas 126 were correctly identified as true negatives. When evaluating the diagnostic efficacy of AMH as a predictor of anovulatory PCOS, with the 21st day progesterone measurement, the following metrics were ascertained: a commendable sensitivity of 94.90%, a robust specificity of 95.45%, a notable positive predictive value of 96.13%, a substantial negative predictive value of 94.03%, and an impressive overall diagnostic accuracy of 95.16%. **Conclusion:** The diagnostic accuracy of Anti-Müllerian hormone in predicting anovulatory PCOS is very high.

Keywords: Anti-Müllerian Hormone, AMH, Polycystic ovarian syndrome, PCOS, Sensitivity

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INTRODUCTION

Polycystic Ovary Syndrome (PCOS) is a common endocrine disorder affecting women of childbearing age, with varying prevalence among different ethnic groups and age ranges. British women aged 20–25 years have a 33% prevalence[1], while Finnish women under 36 have 21.6%[2]. South Asian, particularly Pakistani women, have a striking 52% prevalence, surpassing white populations in the UK, which range from 20% to 25%.[3]

The Rotterdam criteria states that diagnosis, requiring at least two of these: anovulation or oligo-, hyperandrogenism, and polycystic ovaries.[4] PCOS can be categorized into four types based on historical records and physical examinations: Complete PCOS, polycystic ovaries plus anovulation/oligo-, polycystic ovaries plus hyperandrogenism, and anovulation/oligo- plus hyperandrogenism.[5] Clinical manifestations include hyperandrogenism, insulin resistance, and sarcopenic obesity. Insulin resistance and hyperinsulinemia contribute to hyperandrogenism, increasing the risk of gestational diabetes, pregnancy-induced hypertension, preterm birth, and complications during pregnancy.[6]

PCOS often leads to elevated serum Anti-Müllerian Hormone (AMH) levels due to multiple active antral follicles. The use of AMH as a diagnostic indicator remains controversial, with some suggesting an AMH level >3.8–5 ng/mL. Combining the Rotterdam criteria and AMH levels can aid in early and accurate diagnosis.[7]

Approximately 60% of PCOS women have elevated AMH values, associated with reduced pregnancy success rates during controlled intrauterine insemination cycles.[8] The presence of two out of three clinical attributes (AMH, Hyperandrogenism, and Oligo-anovulation) demonstrates strong sensitivity (96%) and specificity (100%) in diagnosing PCOS based on the Rotterdam criteria.[9]

The use of AMH levels as a diagnostic marker for early detection of PCOS, particularly among unmarried women of reproductive age group in Pakistan has garnered significant attention as compared to transvaginal ultrasonogram. This study aims to elucidate the patterns and practicality of serum AMH levels as a supplementary diagnostic tool within the context of the Rotterdam criteria for women diagnosed with PCOS.

MATERIAL AND METHODS

This was a cross-sectional validation study done at the Department of Pathology, Bahawal Victoria Hospital, Bahawalpur from 11 Aug 2021 to 10 Feb 2022. It involved a cohort of 289 female individuals aged 15–30 years, with a diagnosis of PCOS. The sample size was determined as 289 through OpenEpi online software, with a 95% confidence interval, a margin of error at

6.3%, a prevalence rate of anovulatory PCOS at 25%, and sensitivity and specificity values of elevated AMH levels for predicting anovulatory PCOS at 92% and 97% respectively, using a non-probability, consecutive sampling method. Individuals with Cushing's disease, chronic renal failure, hypothyroidism, congenital adrenal hyperplasia, or tumours were excluded from the study.

After approval from Institutional Ethical Review Committee, a total of 289 cases meeting the inclusion criteria were selected, with informed consent. Serum AMH levels were estimated, and a record was maintained regarding the presence or absence of ovulation. The values of AMH levels were subjected to a comparative analysis alongside the results of serum progesterone levels estimated on the 21st day of the menstrual cycle. Data including weight, age, obesity, height, body mass index (BMI), anovulation as indicated by serum AMH levels, and the presence or absence of anovulation determined by serum 21st day progesterone levels (absent/present) were recorded using a specially designed proforma.

The data were analysed using SPSS-25. Mean and standard deviation were computed for variables such as age, duration of PCOS, height, weight, BMI, serum AMH level, and serum 21st day progesterone levels. Parameters such as obesity and the presence or absence of anovulation, both based on serum AMH and serum 21st day progesterone levels, were given as percentages and frequencies. Specificity, sensitivity, negative predictive value, positive predictive value, and the diagnostic accuracy of elevated Anti-Müllerian hormone in predicting anovulation in PCOS were calculated using 2×2 contingency table. Stratification was conducted for variables such as age, BMI, and obesity, with diagnostic accuracy subsequently calculated after stratification.

RESULTS

The age range of the patients spanned from 15 to 30 years, with a mean age of 25.52±2.55 years. The majority (200, 69.2%) of the patients were 15 to 25 years of age. The average height among the participants was 154.33±11.23 Cm, and the mean weight was 71.24±8.98 Kg. The BMI was calculated as 28.99±3.47 Kg/m². The average duration of PCOS was 8.93±4.32 months. Mean concentration of Anti-Müllerian Hormone was 41.55 pmol/L, and the mean levels of 21st day progesterone measured 8.25 ng/mL.

Among patients who 's serum AMH was more than cut-off (4.9 ng/ml), a total of 149 individuals were correctly identified as true positive, while 6 were inaccurately classified as false positive. In contrast, within the group of patients who tested negative for AMH, 8 were erroneously categorized as false negative, while 126 were accurately identified as true negative.

Evaluation of the diagnostic capability of Anti-Müllerian Hormone produced a sensitivity of 94.90%, specificity of 95.45%, positive predictive value of 96.13%, negative predictive value of 94.03%, and an overall diagnostic accuracy of 95.16%.

Table-1: Stratification of diagnostic accuracy

Variables	AMH Level*	21st day progesterone level		*p*
		Positive	Negative	
Anti-Müllerian hormone diagnostic accuracy in predicting anovulatory PCOS, keeping 21st day progesterone as gold standard of ovulation	>cut-off	149 (TP)	6 (FP)	0.0001
	<cut-off	8 (FN)	126 (TN)	
Diagnostic accuracy in the context of the age group 15–25 years (n=200)	>cut-off	106 (TP)	3 (FP)	0.001
	<cut-off	6 (FN)	85 (TN)	
Diagnostic accuracy in the context of the age group 26–30 years (n=89)	>cut-off	43 (TP)	3 (FP)	0.001
	<cut-off	2 (FN)	41 (TN)	
Diagnostic accuracy in the context of the BMI ≤30 Kg/m² (n=186)	>cut-off	103 (TP)	6 (FP)	0.001
	<cut-off	3 (FN)	74 (TN)	
Diagnostic accuracy with respect to BMI ≤30 Kg/m² (n=103)	>cut-off	46 (TP)	0 (FP)	0.001
	<cut-off	5 (FN)	52 (TN)	
Diagnostic accuracy in the context of the non-obese (n=186)	>cut-off	103 (TP)	6 (FP)	0.001
	<cut-off	3 (FN)	74 (TN)	
Diagnostic accuracy with respect to obese (n=103)	>cut-off	46 (TP)	0 (FP)	0.001
	<cut-off	5 (FN)	52 (TN)	

*cut-off value for AMH=4.9 ng/ml

Table-2: Anti-Müllerian Hormone diagnostic accuracy in predicting anovulatory PCOS, keeping 21st day progesterone as gold standard

Variable	Sensitivity	Specificity	PPV	NPV	Diagnostic Accuracy
Anti-Müllerian Hormone diagnostic accuracy in predicting anovulatory PCOS	94.90%	95.45%	96.13%	94.03%	95.16%
Diagnostic accuracy in age group 15–25 years (n=200)	94.64%	96.59%	97.25%	93.41%	95.50%
Diagnostic accuracy in age group 26–30 years (n=89)	95.56%	93.18%	93.48%	95.35%	94.38%
Diagnostic accuracy in BMI ≤30 Kg/m² (n=186)	97.17%	92.50%	94.50%	96.10%	95.16%
Diagnostic accuracy with respect to BMI ≤30 Kg/m² (n=103)	90.20%	100.0%	100.0%	91.23%	95.15%
Diagnostic accuracy in non-obese (n=186)	97.17%	92.50%	94.50%	96.10%	95.16%
Diagnostic accuracy in obese (n=103)	90.20%	100.0%	100.0%	91.23%	95.15%

DISCUSSION

This study was undertaken to assess the diagnostic precision of Anti-Müllerian Hormone in predicting anovulatory PCOS, with the 21st day progesterone measurement serving as the reference standard. The study revealed that AMH exhibited a sensitivity of 94.90%, specificity of 95.45%, positive predictive value of 96.13%, negative predictive value of 94.03%, and an overall diagnostic accuracy of 95.16% in predicting anovulatory PCOS. In another study[10], prevalence of anovulatory PCOS was established as 25%, and the sensitivity and specificity of elevated AMH levels in forecasting anovulatory PCOS were 92% and 97% respectively. Pigny *et al*[11] reported that serum AMH measurement achieved a specificity of 92% and sensitivity of 67%. Lin *et al*[12] identified a cut-off AMH level of 7.3 ng/mL, which conferred a specificity of 76% and sensitivity of 70% for predicting PCOS. The strong correlation between AMH and Antral Follicle Count (AFC) has prompted some researchers to compare their performance in the diagnosis of PCOS.[12] Butt *et al*[13] showed. AMH levels tend to increase with weight, menstrual abnormalities, and hirsutism. LH was the only reproductive hormone that increased with the elevation of serum AMH levels among PCOS women.[13]

Nonetheless, the findings within the literature exhibit a lack of uniformity. A portion of this variation arises from the absence of a clearly defined study population.[14] Notably, some researchers have adhered to the PCOS definition established during the Rotterdam Conference of 2003, which specifies the presence of 12 follicles measuring 2–9 mm in diameter per ovary as the criterion for diagnosing polycystic ovary morphology (PCOM).[15] It is crucial to acknowledge that this specific cut-off is heavily reliant on the quality of ultrasound equipment and the skill of the operator, as observed by Dewailly *et al*[8]. Consequently, with the introduction of more advanced ultrasound technologies and equipment in recent times, this threshold has undergone modifications and can now range from 19 to 25 follicle per ovary. This threshold is likely to continue evolving with the ongoing development of ultrasound technologies.[16] Significant issues pertain to the criteria for including or excluding specific populations from the normative reference group, contributing to the observed heterogeneity in the results.[17] Lastly, technical concerns related to serum Anti-Müllerian Hormone assays further contribute to the variability in the literature. Consequently, to date, it remains unfeasible to establish a universally accepted and unanimous diagnostic threshold for serum AMH in the prediction of PCOS.[18] However, we have found that a threshold of 35 pmol/L or 4.9 (ng/mL) using the enzyme immunoassay AMH-EIA exhibits a commendable specificity and sensitivity of 97% and 92% respectively when compared to Antral Follicle Count (AFC) in predicting PCOs. This outcome was achieved after excluding women with asymptomatic Polycystic Ovaries (PCO) from the control group through the application of cluster analysis.[19]

Pigny *et al*[11] have undertaken a comparison of the five different serum Anti-Müllerian Hormone assays, as described previously, for the purpose of diagnosing PCOS. Their recommendations include a higher cut-off of 5.6 ng/mL or 40 pmol/L when employing manual ELISA assays. This particular threshold is considered biologically indicative of Polycystic Ovary Morphology (PCOM) and corresponds to the 95th percentile of individuals classified as 'pure' controls. In addition, they have suggested a threshold of 4.2 ng/mL (30 pmol/L) for the automatic assays.[11]

If these findings are subsequently validated with the application of new automated serum AMH assays or an ultrasensitive assay, it is conceivable that a heightened serum AMH level could emerge as a dependable and precise marker for PCOM. This could potentially supplant Antral Follicle Count (AFC), which is also a subject of significant debate in the scientific literature.[20] The level of serum Anti-Müllerian Hormone is also associated with the intensity of symptoms in Polycystic Ovary Syndrome, and it tends to be higher when hyperandrogenism or oligo-anovulation is present.[21] Through a principal component analysis, it has been demonstrated that a markedly elevated serum AMH level can serve as an indicator of hyperandrogenism and could help harmonize the diverse PCOS classifications currently in use.[22]

In the case of adolescents and young women diagnosed with PCOS, assessing the ovaries via ultrasonography can sometimes be a challenging task. In such scenarios, serum AMH assays emerge as a viable alternative, a recommendation put forth by the American Association of Clinical Endocrinologists.

CONCLUSION

Anti-Müllerian Hormone exhibits a notably high level of diagnostic accuracy in the prediction of anovulatory Polycystic Ovary Syndrome. It is recommended that Anti-Müllerian Hormone be employed as the primary diagnostic test for anticipating anovulation in PCOS and ultimately contributing to a reduction in the morbidity experienced by these specific patients.

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Address for Correspondence:

Dr Ammar Bin Saad, Assistant Professor Haematology, Department of Pathology, Ayub Medical College, Abbottabad, Pakistan. **Cell:** +92-311-4549201

Email: ammarbinsaad1@yahoo.com

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Contribution of Authors:

AG: Conception and Design of work
RS: Design of work, Acquisitions and Analysis
IS: Interpretation and Drafting
HAH: Final Approval and Agreement to be accountable for all aspects of work
SK: Resulting and Interpretation
ABS: Drafting and Acquisitions and Analysis

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ORIGINAL ARTICLE:

COMPARISON OF INTRAVENOUS DIAZEPAM AND INTRAVENOUS MIDAZOLAM FOR TREATMENT OF RECURRENT FEBRILE SEIZURES

Waseem Pasha, Awais Amjad*, Imran Ahmed, Usman Nawaz***, Muhammad Usman Sajid**, Muhammad Usman Ali*, Seemi Habib†**

Department of Paediatrics, *Physiology, **Medicine, ***Pharmacology, CMH Kharian Medical College, †Paediatrics Unit, THQ Hospital, Kharian, Pakistan

Background: Recurrent febrile seizures are seizures in children less than 5 years of age which reoccur within 24 hours. Benzodiazepines such as midazolam and diazepam are used to stop and decrease the recurrent rate of febrile seizures. The objective of study was to compare the efficacy of intravenous midazolam with intravenous diazepam for treatment of recurrent febrile seizures in paediatric patients. **Methods:** This observational cross-sectional study observed 60 children aged 6 to 59 months with recurrent febrile seizures who presented at paediatric clinic in Combined Military Hospital Kharian and THQ Hospital, Kharian from Sep to Nov 2023. The response time was noted for children who received intravenous midazolam 0.2 mg/Kg only and IV diazepam 0.2 mg/Kg only. The children were randomly allocated into group A (midazolam group) and Group B (diazepam group.) Study was done after taking informed consent from the parents. Both drugs were given during the fits. **Results:** The time interval from drug administration to cessation of febrile seizures was 2.50±0.94 minutes in the midazolam group and 2.4±1.12 minutes in the diazepam group. There was no significant difference between the two groups (p=0.567). Minor dizziness and sedation were the only main effects reported. **Conclusion:** Both diazepam and midazolam have same efficacy in the treatment of recurrent febrile seizures.

Keywords: Midazolam, Diazepam, Febrile seizures, Efficacy

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INTRODUCTION

A seizure episode in a child six months to fifty-nine months of age plus low-grade fever, without central nervous system infection or metabolic imbalance, is called a febrile seizure.[1] It is generalized tonic-clonic type of seizure lasting usually for 15 minutes. It stops itself after some time. Simple febrile seizures are common in children, occurring in 3–6% of children. Simple febrile seizures patients do not require hospital admission. Common risk factors of simple febrile seizure are viral illness and vaccinations.[2]

On the other hand a complex recurrent febrile seizure lasts more than 15 minutes, is focal and reoccurs within 24 hours. They are not likely to stop themselves, and a benzodiazepine should be injected to stop the complex recurrent seizure. If seizures are not stopped, it can lead to brain damage. Rapid control of seizures reduces future adverse outcomes.[3] Complex recurrent febrile seizure paediatric patients require hospital admission sometimes, but children of younger age and unreliable follow-up must be admitted.[4] Risk factors of recurrent febrile seizures include genetics, age less than 17 months, fever duration less than 24 hours, low grade fever, first degree relative, children in day care centre, dehydrated children and male gender.[5] Only 2% children with this type of seizures might have an attack of epilepsy in future.[6] Seizures cause emotional and mental stress for parents and treating doctors. Parents are very terrified when their children get recurrent seizures. There is risk of injury with a seizure lasting more than 15 minutes.[7]

Many medicines have been used including diazepam, phenobarbitone, midazolam, zinc, paracetamol have been used to reduce the recurrence of febrile seizures.[8] Phenobarbitone is an antiepileptic drug and has side effects and routine use is not recommended. Intramuscular approach (IM) for diazepam and midazolam causes persistent pain at injections site which makes children uncomfortable. Per Rectal (PR) administration of diazepam is unpleasant for paediatric patients. Disrobing children makes them more uncomfortable. Buccal route is also difficult in patients with seizures because of risk of finger biting. IV route is the preferred route. IV diazepam is the most commonly used benzodiazepine; however, it has rare side effects of respiratory depression, bradycardia and respiratory arrest.[9] Midazolam is water soluble, fast acting, slightly expensive, not widely used in Pakistan yet. Its ring structure closes at physiological *p*H, becomes lipophilic and passes blood-brain-barrier rapidly. It has fast clinical effects on the central nervous system. No respiratory side effects are reported with midazolam. Intranasal midazolam is becoming very popular for the treatment of febrile seizures.[10]

Antipyretics can decrease the discomfort of the child but do not reduce the frequency of recurrent seizures.[11] A study found no benefit of zinc supplementation for prevention of recurrent febrile seizures.[8]

Our study aims to compare intravenous midazolam with diazepam in paediatric patients of Kharian for management of recurrent febrile seizures.

MATERIAL AND METHODS

This observational cross-sectional study was carried out at CMH Kharian and THQ Hospital Kharian from September to November 2023, after getting ethical approval from CMH Kharian Medical College Ethical Committee (File No. 2000/Gen/ECA/2023/01). Sample size was calculated using WHO Sample Size Calculator at confidence level of 95%, alpha error of 5%, and study power of 80%.[12] Total 60 children of either gender were enrolled through non-probability sampling technique. Inclusion criteria was children between ages of 6 months to 59 months presenting with recurrent febrile seizures. Exclusion criteria was presence of trauma to CNS, hypoglycaemic children, hypocalcemic fits, and known epileptic children. The response time for seizure cessation was noted for children who received IV midazolam 0.2 mg/dL only and IV diazepam 0.2 mg/dL only. Group A (midazolam group) had 32 children. Group B (diazepam group) had 28 children. Informed consent from the parents were obtained. Both drugs were given only during the fits.

Data was analysed using SPSS-20. Variables such as age and time were presented as mean with standard deviations. Independent sample *t*-test was used to compare the mean of both groups, and $p \leq 0.05$ was considered significant.

RESULTS

Among 60 enrolled patients there were 32 males and 28 females. The mean age of patients in Group A was 3.27±1.31 years and in Group B it was 3.77±1.09 years. There was no significant difference between age of both groups. The time interval between drug administration and cessation of seizure was similar for both IV midazolam and IV diazepam. No significant side effects were reported in either group.

Table-1: Clinical characteristics of study groups

	IV Midazolam	IV Diazepam
Male	16	16
Female	15	13
Mean age (years)	3.27±1.31	3.77±1.09
Cause of febrile seizures		
URTI	6	7
Acute otitis media	8	9
Bronchopneumonia	6	6
Dysentery	4	3
Other	5	6

Table-2: Drug effect time interval among study groups

Time interval (min)	IV Midazolam Mean±SD	IV Diazepam Mean±SD	*P*
Drug administration to cessation of seizure	2.50±0.94	2.4±1.12	0.567

DISCUSSION

In our study URTI was the most common cause of febrile seizure, followed by acute otitis media, bronchopneumonia and dysentery. The underlying patho-physiological causes of recurrent febrile seizures are diverse. Any pathology that can disrupt the normal neuronal function and connectivity can make the brain epileptic. Gene mutations in voltage gated channels of sodium, calcium, potassium and chloride can lead to increased excitability in neurons. There is loss of functions of inhibitory GABA interneurons.[12] However, a clear aetiology is still lacking.

We found that intravenous midazolam was just as effective at reducing seizure activity of febrile seizures as intravenous diazepam. Hence intravenous midazolam can be used as an alternate remedy to diazepam.

Similarly, study done by Batool *et al*[13] in Fauji Foundation Hospital Pakistan showed that intranasal midazolam and intravenous diazepam had same efficacy. They had 62 patients from age 3 months to 12 years and used intranasal midazolam. We used intravenous midazolam in patients aged 6 months to 5 years. However, Kazmi *et al*[14] study in neurology unit of Children Hospital, Lahore Pakistan on 164 paediatric patients showed that intravenous midazolam was better than intravenous diazepam in managing status epilepticus. In Kazmi study patients were suffering from status epilepticus, and our study patients had recurrent febrile seizures. A Swiss study[15] reported that IV diazepam is better in adults and intranasal midazolam is better in children for termination of seizures.[15]

CONCLUSION

Intravenous midazolam can be used as a rescue medication for the treatment of recurrent febrile seizures in children in the absence or shortage of diazepam. Both midazolam and diazepam have same efficacy.

LIMITATIONS

This study did not study neonates. Further studies on larger children population with other types of seizures are recommended.

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Address for Correspondence:

Dr Waseem Pasha, Assistant Professor Paediatrics, CMH Kharian Medical College, Kharian, Pakistan. **Cell:** +92-333-9275722

Email: waseempashaqureshi@yahoo.com

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Contribution of Authors:

WP: Concept and design of study, Getting ethical approval, Data collection, Drafting of article, Critical review
AA: Concept and design of study, Data collection, Drafting of article, Critical review
IA: Data collection, Drafting of article, Critical review, Statistical analysis
UN: Data collection, Drafting of article, Critical review, Statistical analysis
MUS: Data collection, Drafting of article, Critical review, Statistical analysis
MUA: Drafting of article, Critical review
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ORIGINAL ARTICLE

INTEGRIN BETA2 GENE VARIANT CAUSING LEUKOCYTE ADHESION DEFICIENCY TYPE 1 IN A PAKISTANI FAMILY

Rabea Nasir, Sheeba Shabbir*, Lubna Siddique, Shahida Awais***, Zara Khalid†, Ambreen Zahoor††, Zahid Azeem†††, Farzana Majeed‡**

Department of Physiology, M. Islam Medical College Gujranwala, *Department of Forensic Sciences, HBS Medical & Dental College, **Department of Physiology, Rawal Institute of Health Sciences, ***Fazaia Medical College, †Department of Biochemistry, Rawal Institute of Health Sciences, ††Department of Medicine, HBS Medical & Dental College, Islamabad, †††Department of Biochemistry, AJK Medical College, Muzaffarabad, ‡Department of Physiology, HBS Medical & Dental College, Islamabad, Pakistan

Background: Leukocyte adhesion deficiency type 1 (LAD1), an autosomal recessive condition, arises from partial or complete deficiency of CD18 expression. LAD1 patients commonly manifest recurrent skin and respiratory tract infections, delayed umbilical cord separation, and impaired wound healing due to hindered leukocyte migration. This study aims to clinically and molecularly diagnose LAD in a highly consanguineous Pakistani family, investigating a recurrent mutation within the integrin β2 (*ITGB2*) gene. **Methods:** A comprehensive clinical and molecular diagnosis of LAD1 was made in on a patient from a consanguineous Pakistani family. Lymphocyte subset analysis was performed using a flow cytometer, followed by whole exome sequencing and DNA Sanger sequencing to identify the pathogenic mutation within the *ITGB2* gene. Further to provide genetic counselling all the healthy siblings were also Sanger sequenced. **Results:** Flow cytometry indicated CD18 deficiency, while sequencing of the *ITGB2* gene unveiled a nonsense mutation, c.186C>A, p. (Cys62*), located in exon four. This mutation segregates in an autosomal recessive pattern within the family. **Conclusion:** A mutation c.186C>A (Cys62*) in a patient of LAD1 was identified which is potentially pathogenic in nature.

Keywords: Genetic study, Whole exome sequencing, Sanger sequencing, Flow cytometry, Leukocyte adhesion deficiency

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INTRODUCTION

Leukocyte adhesion deficiency type 1 (LAD1; OMIM #116920) is a rare (1 in 100,000 live births) autosomal recessive type of inherited disorder. Common manifestations include repeated bacterial and fungal infections including skin and respiratory tract infections, delayed wound healing, skin ulcers, and sepsis and otitis media owing to the barrier of leukocyte migration to the site of infection.[1] The hallmark features include delayed separation of the umbilical cord and elevated white blood cells (leukocytosis).[1–3]

The first case of LAD1 was reported back in 1980 and till now more than 300 patients have been reported worldwide.[3] More than 26 cases have been reported only from Pakistan. Over 80 homozygous mutations in Integrin β2 (*ITGB2*) gene have been reported producing a variety of different LAD1 phenotypes including severe (<2% expression of β2 integrins) or moderate (2–30% expression of β2 integrins).[4] Mutations in the gene *ITGB2* (OMIM, 116920) have been reported to cause LAD1. The gene is localized on 21q22.3 comprising 16 exons, which encode 769 amino acids (approximately 85~95 KD) β2 integrin protein (CD18).[5,6] The protein CD18 on the surface of leukocytes is involved in adhesion and transmigration. Patients with impaired functional CD18 suffer from LAD1, where accumulation of myeloid leukocytes is observed at extravascular sites. Therefore elevated levels of leukocytes, neutrophils, and recurrent infections are observed due to the inability to recruit leukocytes at the site of infections.[7–9]

Disease diagnosis is based on analysis of CD18 expression on the surface of leukocytes using flow cytometry.[7,10] Based on the CD18 expression disease can be grouped into moderate with 2–30% and severe with <2%. Patients suffering from moderate levels of CD18 expression survive early childhood with proper antibiotics while patients suffering from a severe type of LAD1 succumbed to infections.[3]

Early diagnosis should be considered in every patient with recurrent infection and a markedly increased leukocyte count. Physicians should opt for flow-cytometery for an early diagnosis in patients with repeated infections, marked leukocytosis, and delayed umbilical cord separation. To find the exact defect at the molecular level *ITGB2* gene is Sanger Sequenced.[3,11] A molecular diagnosis approach including Sanger sequencing is highly recommended to define the precise molecular defect in the β2 subunit.[3,12]

According to (Medline plus.gov and Human Genome Mutation Data Bank, HGMD) at least 90 different *ITGB2* gene mutations have been identified casing LAD1.[13] The mutations include missense, nonsense, splice site, and deletion mutations. The majority of these mutations fall in exons 5 to 9 that encode the β1 domain of ITGB2. The present study reveals a clinical and molecular diagnosis of LAD1 in a

6-month-old male from a highly consanguine Pakistani family. DNA Sanger sequencing identified a nonsense mutation (p.Cys62*).

METHODOLOGY

In this project, we enrolled a 6-month-old boy suffering from LAD1, born to a highly consanguine family from a remote area of Punjab, Pakistan. The patient was admitted to Aga Khan University Hospital, Karachi, Pakistan. The patient was thoroughly examined for infections such as gastrointestinal tract (diarrhoea) skin (omphalitis) and respiratory tract (sinusitis, tonsillitis, otitis media, pharyngitis, and pneumonia) by an expert team of paediatricians in the Department of Paediatrics, Aga Khan University Hospital.

Flow cytometry was performed to check the status of the lymphocyte subset in patients at the Armed Forces Institute of Pathology, Department of Immunology (AFIP-Immunology), Rawalpindi. Under the supervision of an expert immunologist detailed clinical history was collected for immune deficiency workup. The genetic analysis including, polymerase chain reaction and DNA sequencing, was carried out at the Biological Sciences Department, Quaid-i-Azam University, Islamabad, Pakistan. To have permission to publish the research findings written signed consent was obtained from parents. Study approval was granted by the Institutional Review Board of HBS Medical and Dental College, Islamabad.

Based on clinical history patient was suspected of having LAD1. To perform immunological (flow cytometry) and haematological test (blood complete picture) studies whole blood samples of approximately 5 mL were drawn into EDTA tubes. Flow cytometry and blood complete picture were performed on an automated Sysmex KX21 Hematology Analyzer (Sysmex Corporation, Japan). Lymphocyte subset analysis was performed on a FACSanto II machine (Becton Dickinson, USA) using CF11bPE, CD18FITC, and anti-CD11C antibodies. Processed patient samples by the addition of required RBC lysis (stain/lyse/wash procedure) proper washing with recommended antibodies and were taken to flow cytometer and analysed using BD software of FACSDiva. LAD1 classification CD18 concentration as (moderate >2%) and severe (<2%) were applied to the patient's sample.[3] European Society for Immunodeficiencies (ESID) probable criteria were used for establishing LAD1.[10]

To perform genetic analysis whole blood samples (3–5 mL) were collected from the patient, parents, and available healthy siblings. Using QIAamp® DNA Mini Kit (Hilden Germany), DNA was extracted from available blood and was quantified on a Nanodrop1000 spectrophotometer (Thermal Scientific, Wilmington, MA, USA). Using Primer-3 software (http://bioinfo.ut.ee/primer3-0.4.0/) *ITGB2* gene exon-specific primers were constructed. Using standard PCR protocol[14] each *ITGB2* protein-coding exons was PCR amplified. The qualitative analysis of PCR amplified products (3 µL) was performed by ethidium bromide-stained 2% agarose gel electrophoresis under a UV illuminator. Later, PCR-amplified products were purified using a commercially available kit (Axygen, CA, USA). Purified PCR products were subjected to Sanger Sequencing using BigDye Terminator v-3.1 Cycle Sequencing Kit on Beckman Coulter CEQ-8000 Analyzer (Stanwood, Washington, USA).

DNA Sanger sequencing files for each exon of the *ITGB2* gene were analysed by comparing them with corresponding control gene sequences obtained from the Ensemble Genome Browser database (http://ensembl.org/index.html). To nucleotide sequence variant BioEdit sequence alignment editor version 6.0.7 was used. The pathogenicity score of the identified variants was measured using MutationTaster (http://www.mutationtaster.org/), and Polymorphism Phenotyping V2 (PolyPhen 2).

RESULTS

The patient enrolled in the current study belonged to a highly consanguine family from Punjab (Figure-1A). On admission, the patient was anaemic with a fever of 39 °C. Detailed clinical examination by the expert team of physicians revealed that the patient was suffering from respiratory tract and skin infections. There had been a delay in umbilical cord separation for more than two weeks. The detachment site of the umbilical cord and site of skin infection was devoid of pus formation (Table-1). At the age of 2 months abscess developed behind the left ear which was drained two times along with on-and-off fever and vomiting. Skin abscess behind left ear was observed at the age of 2 months which was drained two times. The patient had on and off fever with vomiting. Ultrasound revealed no sign of lymphadenopathy or hepatosplenomegaly. Other examinations including cardiovascular and nervous systems remained unremarkable.

Figure-1A shows pedigree of the family suffering from LAD1. Circles show females while squares show males. Double lines between I-1 and I-2 show consanguine marriage. Filled circle (II-3) shows patient. Figure-1B shows a chromatogram obtained through Sanger sequencing. Arrowhead showing the position of *ITGB2* exons 5 mutation [c.186C>A, p.(Cys62*)] in the patient (II-1), carrier (Father I-2), and healthy sibling (II-3).

The LAD1 diagnosis was done through clinical tests such as immune functions and flow cytometer. The patient's immunoglobulin levels (IgG, IgM, and IgA) are shown in Table-1. Blood complete picture test revealed leukocytosis compared to relative

neutrophilia. Blood complete picture test revealed white blood cell (11.7×10^3/L), platelets count (338×10^9/L), lymphocyte (13.5%), neutrophils (84.7%), and CRP (56.60 mg/dL). Similarly, lymphocyte subset analysis revealed raised T, B and NK cells (Table-1). Flow-cytometry based Dihydro rhodamine (DHR) assay and nitroblue tetrazolium slide test (NBT) were negative for neutrophil function. Flow cytometry was performed which clearly showed a reduced level of CD18 expression (Figure-2). These findings were consistent with leukocyte adhesion deficiency type I (LAD1) disease.

Whole exome sequencing (WES) identified two homozygous variants in genes (*NCF2*) on chromosome 1 and *ITGB2* on chromosome 21. Besides homozygous mutations, heterozygous variants in 5 other genes were also identified (Figure-1). Based on laboratory findings which were consistent with LAD1 deficiency the underlying disease-causing gene *ITGB2* was completely Sanger sequenced which revealed a non-sense mutation [c.186C>A, p.(Cys62*)]. This non-sense mutation was found to be completely segregating in the family including parents as (heterozygous) and healthy siblings (Figure-1).

Table-1: Patient blood complete picture and immunological tests

Name of Test	Patient value	Reference Value
Total Leucocyte Count	16.7×10^3 cells/µL	$4–15\times10^3$ cells/µL
Haemoglobin level	12.7 g/dL	11.1–16.3 g/dL
Lymphocytes	13.5% (12.1×10^3/µL)	20–40% ($1–3\times10^3$ cells/µL)
Neutrophils	83.7% (97.5×10^3/µL)	40–80% ($2–7\times10^3$ cells/µL)
Platelets	273×10^3/µL	$150–410\times10^3$ cells/µL
Monocytes	7% (8.3×10^3/µL)	2–10% ($0.2–1\times10^3$ cells/µL)
Immunological Work-up		**Reference range**
Serum Immunoglobulins (Ig)		
IgG	8.5	2.3–14.1 g/L
IgA	0.19	0–0.83 g/L
IgM	1.10	0–1.45 g/L
IgE	165	<100 IU/mL
Neutrophil Function test		
NBT[1] slide test	No abnormality detected	
Dihydrorhodamine Test	Stimulation Index (SI)=400 (Control=200)	
C-Reactive Protein	56.6 mg/dL	0.9 mg/dL
Flow cytometric analysis for CD11/CD18	Expression of CD11b, CD11c, and CD18 was less than 1% on the patient's neutrophils	
C-reactive Protein-H	35 mg/dL	< 0.744 mg/dL

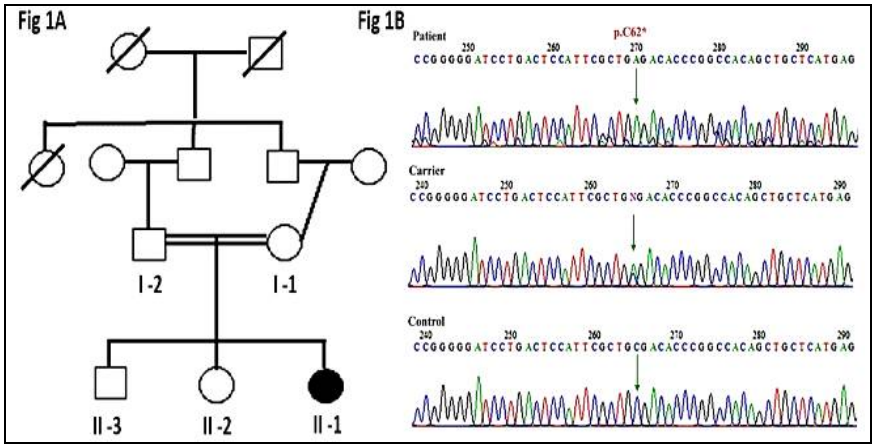

Figure-1: Pedigree and *ITGB2* Sequencing

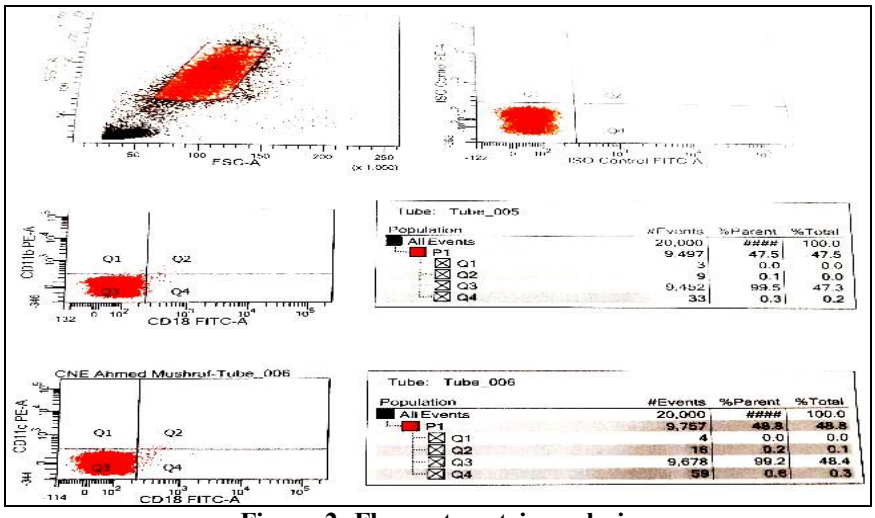

Figure-2: Flow cytometric analysis

The photograph is showing absence of (<1%) CD11b, CD11c and Cd18 on granulocytes

DISCUSSION

LAD1 is the most common leukocyte adhesion deficiency and mutations in the Integrin Subunit Beta-2 gene (*ITGB2*) have been reported causing LAD1 in more than 300 cases worldwide. Leukocyte adhesion deficiency is characterized by recurrent skin and respiratory tract infection with delayed umbilical cord separation.[1–3]

LAD1-specific diagnosis was established by deficiency of integrin subunit of β-2 (CD18) expression on the leukocyte surface. Based on the concentration of CD18 expression in leukocytes, the severity of the LAD1 disease can be established.[3] LAD1 patient's blood complete picture test presents leukocytosis and neutrophilia. CD18 molecules allow neutrophils to move out of the bloodstream to migrate to the site of infection. Those with partial reduction in CD18 expression exhibit less severe symptoms and can survive till adulthood.[6]

In the present study, we enrolled a 6-month-old male patient from remote village of Punjab, Pakistan. The patient succumbed to repeated respiratory tract infections and delayed (more than 2 weeks) umbilical cord separation and expired in 8th month soon after initial diagnosis. These clinical manifestations were coherent with earlier studies in other populations.[3,4,15]

Flow cytometry in the case presented in the current study revealed (<2% CD18) molecules, whole exome sequencing identified previously reported nonsense mutation [p. (Cys62*)] and its segregation in the Tri-ADD was confirmed through Sanger sequencing.[14] This non-sense mutation is located at the tightly folded N-terminal extracellular domain of the integrin β2 subunit. This β2 integrin extracellular domain is involved in communication between α and β subunits.[3,16]

This study presents detailed clinical features in patients suffering from LAD1 from a consanguine family. Flow cytometry revealed the severe type of leukocyte adhesion deficiency while WES identified a non-sense mutation in the *ITGB2* gene. The clinical and laboratory findings are compatible with those reported earlier. This study will not only help in screening patients with recurrent skin infections and neutrophilia for LAD1 disorder but will also facilitate genetic counselling in the Pakistani population.

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Address for Correspondence:

Prof Dr Farzana Majeed, Department of Physiology, HBS Medical & Dental College, Shaheed Zulfiqar Ali Bhutto Medical University, Islamabad, Pakistan. **Cell:** +92-334-5597265

Email: drfarzanajaved@hotmail.com

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Contribution of Authors:

RN: Manuscript writing, data analysis
SS: Laboratory data acquisition and analysis
LS: Data formatting interpretation
SA: Literature Review and data analysis
ZK: Laboratory work and data analysis
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ORIGINAL ARTICLE

PREVALENCE OF DYSLIPIDAEMIA AMONG PATIENTS OF POLYCYSTIC OVARIAN SYNDROME: A CROSS SECTIONAL ANALYTICAL STUDY

Saleha Zafar, Tabinda Yasmin*, Rabia Saeed, Javaria Aslam***, Tanzila Rafiq†, Ammar Bin Saad††**

Department of Pathology, Quaid-e-Azam Medical College, Bahawalpur, *Nawab Sir Sadiq Hospital, Bahawalpur, **CMH Institute of Medical Sciences Bahawalpur, ***Department of Medicine, Quaid-e-Azam Medical College, Bahawalpur, †Department of Obstetrics and Gynaecology, CMH Institute of Medical Sciences, Bahawalpur, ††Department of Pathology, Ayub Medical College, Abbottabad, Pakistan

Background: Polycystic ovarian disease is a genetically complicated condition, most prevalent in women of reproductive age group. It is strongly associated with obesity, dyslipidaemia s, and menstrual disorders. Objective of this study was to determine the prevalence of dyslipidaemia in patients of polycystic ovarian syndrome (PCOS). **Method:** A cross-sectional analytical study was conducted at the Department of Gynaecology, Bahawal Victoria Hospital, Bahawalpur, from 1st Jan to 31st Dec 2021. All newly diagnosed patients of PCOS between the ages of 18 and 35 with a BMI <25 Kg/m^2 were included in the study. Participants were divided into subgroups according to their age and BMI. The fasting lipid profile was measured through a blood sample taken from each patient after an overnight fast. Total cholesterol >200 mg/dL, LDL-C >130 mg/dL, Triglycerides >150 mg/dL, and HDL-C <40 mg/dL were labelled as dyslipidaemia. Chi-square test was used to calculate and estimate the relative risk (RR) of any associations observed. **Results:** Among total 286 analysed patients of PCOS, mean age was 24.4±5.367 years and mean BMI was 21.01±1.912 Kg/m^2. Sixty nine (24.13%) patients had dyslipidaemia. Patients in the 18–27 age group and a BMI of 21–23 Kg/m^2 were more likely to have dyslipidaemia with PCOS, [RR 44.90; 95% CI (16.93, 119.04); p<0.001], and [RR 32.12; 95% CI (8.02, 128.61); p<0.001] respectively. **Conclusion:** Dyslipidaemia is seen in 24.13% individuals with PCOS. Screening in routine is recommended.

Keywords: Polycystic ovarian syndrome, PCOS, dyslipidaemia, metabolic syndrome

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most prevalent ovarian condition that is associated with androgen excess in women, which explains the immense attention of endocrinologists. This syndrome can result in clinical hyperandrogenism, biological dysovulation, or even infertility. Global incidence of PCOS is 5 to 18% among women of reproductive age.[1] Although the biochemical mechanism behind PCOS pathogenesis stays widely undefined, accumulating data suggests that hyperandrogenism plays an essential role in PCOS development and sequelae. PCOS patients are more likely to develop metabolic syndrome.[2] There is a considerable risk of cardiovascular disease in PCOS-afflicted women if these risk factors are present.[3] Atherogenic dyslipidemia, raised blood pressure, dysglycaemia, a pro-thrombotic condition, and a pro-inflammatory state make up the metabolic syndrome, which causes atherosclerotic cardiovascular disease (ASCVD). Atherogenic dyslipidaemia raises plasma triglycerides, LDL-C and lowers HDL-C.[4,5] Obesity is a risk factor for both dyslipidaemia and PCOS, hence women with PCOS should be evaluated for dyslipidemia.[6,7]

Dyslipidaemia, defined as elevated total or low density lipoprotein cholesterol (LDL-C) or low levels of high density lipoprotein (HDL-C), is an important risk factor for coronary heart disease and stroke.[8] It can lead to symptomatic coronary artery disease, stroke and peripheral artery disease. High levels of triglycerides (TG) >1,000 mg/dL (>11.3 mmol/L) can cause acute pancreatitis.[7] Statins are the most frequently used drugs to treat dyslipidaemia. By preventing the liver's ability to produce cholesterol, it lowers LDL levels. Proprotein convertase subtilisin/kexin type 9 (PCSK 9) inhibitors and fibrates are among the non-statin medications.[9] Lifestyle adjustments may lower cholesterol and TG. The first step is dietary modification; saturated fat, processed carbohydrates, and alcohol should be reduced. Fruits, vegetables, lean proteins, and whole grains may assist.[9,10]

It is important to diagnose dyslipidaemia in patients with PCOS as early as possible to prevent long term complications of the cardiovascular system. Moreover, early recognition and treatment of dyslipidaemia may also help in the treatment of PCOS.

MATERIAL AND METHODS

After approval from the Ethical Review Board at DME/QAMC Bahawalpur, this cross-sectional analytical study was conducted from 1st Jan 2021 to 31st Dec 2021. Data was collected from patients visiting the

Gynaecology Departments at Bahawal Victoria Hospital, Qauid-e-Azam Medical College, Bahawalpur. Sample size of 300 was calculated with anticipated proportion 24.7%, Confidence Interval 95%, and required precision (d) of 0.05.

All patients in age group of 18–35 years and diagnosed with PCOS on ultrasonography were included in the study after their informed written consent. Demographic information like age and marital status, were noted. Exclusion criteria for the study were those with history of familial hyperlipidaemia, ischemic heart disease, diabetes mellitus, thyroid disease, active smoking, and those taking oral contraceptives, steroids, or lipid-lowering medications. Body Mass Index (BMI) of all participants were noted and those who had BMI >25 Kg/m^2 or <18 Kg/m^2 were excluded from the study.

After an overnight fast, 3 mL blood sample was obtained from each patient and analysed in the Pathology Department of QAMC, Bahawalpur. Total cholesterol, HDL-C, LDL-C and Triglycerides were measured on the sample. All tests were performed on a completely automated chemistry analyzer AU-630, and all levels were measured in mg/dL. Total cholesterol >200 mg/dL, LDL-C >130 mg/dL, Triglycerides TG >150 mg/dL, and HDL-C <40 mg/dL were labelled as dyslipidemia.[11]

The frequency and percentage for the categorical variables were calculated, as were the Mean±SD for the continuous variables. To determine the impact of effect modifiers on the dyslipidaemia, age and BMI stratification was employed. The post-stratification chi-square test was used to determine the relative risk of dyslipidaemia among various age and BMI groups, and $p<0.05$ was considered statistically significant.

RESULTS

Total 286 patients out of 300 met the inclusion criteria, 210 (73.43%) were from age group 18–27 years. Mean age of the patients was 24.40±5.367 years and mean BMI was 21.01±1.912 Kg/m^2, [140 (49%) had BMI 18–20 Kg/m^2 and 146 (51%) had BMI 21–23 Kg/m^2]. Sixty-nine (24.13%) patients had dyslipidaemia. Types of dyslipidaemia among these patients are given in Table-1.

When the participants were divided into different age groups and analysed for the presence or absence of dyslipidaemia, it was found that 65 (85.53%) patients from age group 28–35 years had dyslipidemia. Risk of having dyslipidaemia was greater in this age group when compared with patients of age group 18–27 years [65 (85.53%) *vs* 4 (1.9%); RR 44.90; 95% CI (16.93, 119.04); $p<0.001$]. (Table-2).

When patients of different BMI groups were analysed for the presence or absence of dyslipidaemia and relative risk of having dyslipidaemia was calculated among these group, it was found that patients of BMI 21–23 Kg/m^2 were more likely to have dyslipidaemia as compared to those of BMI 18–20 Kg/m^2 [67 (46%) *vs* 2 (1.43%); RR, 32.12; 95%CI (8.02, 128.61); $p<0.001$]. (Table-3).

Table-1: Types of dyslipidemia in the study group

Type of Dyslipidaemia	Number	Percentage
Total Cholesterol >200 mg/dL	30	43.5
HDL <40 mg/dL	7	10.1
LDL-C >130 mg/dL	5	7.3
Triglycerides >150 mg/dL	27	39.1

Table-2: Age distribution of dyslipidaemia patients

	Dyslipidemia			
Age Group	**Yes (%)**	**No (%)**	**Total**	***p***
18–27 Year	4 (1.9)	206 (98.1)	210 (73.43)	0.000
28–35 Year	65 (85.53)	11 (14.47)	76 (26.57)	
Total	**69 (24.13)**	**217 (75.87)**	**286**	

Table-3: BMI among dyslipidaemia patients

	Dyslipidemia			
BMI Group	**Yes (%)**	**No (%)**	**Total**	***p***
18–20 Kg/m^2	2 (1.43)	138 (98.57)	140 (49)	0.000
21–23 Kg/m^2	67 (46)	79 (54)	146 (51)	
Total	**69 (24.13)**	**217 (75.87)**	**286**	

DISCUSSION

Polycystic ovary syndrome is an emerging reproductive disorder in young girls at pubertal age and most commonly associated with dyslipidemias.[12] A study showed that PCOS is strongly associated with obesity and dyslipidaemias in reproductive age group women.[13] A definitive criteria for diagnosis of hyperlipidaemia is not clearly explained in most of the studies. It is recommended by American College of Obstetricians and Gynaecologists to consider fasting lipid profile for risk assessment of cardiovascular disease in women with PCOS.[14]

Obesity, polycystic ovaries, clinical or biochemical hyperandrogenism, and prolonged anovulation are hallmarks of PCOS. Our results provide evidence of association between deranged lipid profile in reproductive age women. However, Joham AE *et al*[15] revealed that oligomenorrhoea or amenorrhoea is linked to hyperandrogenism, and hirsutism or acne may occur as clinical symptoms. Although several pathophysiologic explanations for the emergence of PCOS have been put forth, insulin resistance (IR) is now recognized as being relevant to the disease. Women with PCOS who have IR have an increased risk of type II diabetes and cardiovascular disease.[16]

Insulin resistance, hyperinsulinemia, and obesity are often linked with a higher risk of developing metabolic syndrome and type 2 diabetes. The metabolic syndrome is a collection of cardiovascular disease-related risk factors. It is typified by central obesity, high TG, LDL cholesterol levels, and insulin resistance. Moini *et al*[17] found that among women of reproductive

age with PCOS, the prevalence of metabolic syndrome was 22.7%, which was comparable to the prevalence of dyslipidaemia among PCOS patients in this study. Women with PCOS have a high prevalence of metabolic syndrome and its individual components such as low HDL levels.

In a study by Kim JJ *et al*[18], 865 consecutive PCOS patients had a mean age of 24.9, a mean BMI of 22.4 Kg/m^2, and frequency of dyslipidaemia as 35.7%. Kim JJ *et al*[19] have reported that in 166 women with PCOS, frequency of raised Triglycerides was 26.7% and frequency of low HDL-C was 30%. Hong Y *et al*[20], found the prevalence of dyslipidaemia in PCOS women as 24.7%, and it was significantly greater in the insulin resistance group compared to the non-insulin resistance group (39.9% *vs* 15.4%, $p<0.05$).

CONCLUSION

Patients with PCOS had a prevalence of dyslipidaemia as 24.13% making them prone to developing metabolic syndrome and hence an increased risk of cardiovascular disease. Patients with polycystic ovarian syndrome should be tested for dyslipidaemia.

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Address for Correspondence:

Dr Ammar Bin Saad, Assistant Professor Haematology, Department of Pathology, Ayub Medical College Abbottabad, Pakistan. **Cell:** +92-311-4549201

Email: ammarbinsaad1@yahoo.com

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Contribution of Authors:

SZ: Design of work, Acquisitions and Analysis
TY: Concept and Design of work
RS: Interpretation and Drafting
JA: Final Approval and Agreement to be accountable for all aspects of work
TR: Results tabulation and Interpretation
ABS: Drafting, Acquisitions and Analysis

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ORIGINAL ARTICLE

POTENTIAL RESTORATION OF FATTY LIVER ENZYMES WITH *STEVIA REBAUDIANA*

Sadia Mubarak, Amna Faruqi*, Saima Latif Qureshi, Tooba Zafar*, Irfan Afzal Mughal, Asma Irfan**

Department of Physiology, Islamabad Medical and Dental College, *NUST School of Health Sciences, **Akhtar Saeed Medical College, Islamabad, Pakistan

Background: Obesity is a prevalent issue affecting a growing number of individuals globally. It is a systemic disorder with complications and co-morbidities. Non-alcoholic fatty liver disease (NAFLD) is a disastrous outcome of metabolic syndrome induced by obesity. Objective of this study was to find the effect of Stevia leaves in restoration of liver function tests (ALT, AST, ALP, and Bilirubin) in obese Sprague Dawley rats. **Methods:** This animal experimental study was carried out in the Physiology Department, in collaboration with the Pathology Department of Islamabad Medical and Dental College, and National Institute of Health, Islamabad on 90 healthy male Sprague Dawley rats over a period of 14 weeks. The animals were divided randomly into three groups of 30 rats each. Group 1 was given normal diet while Groups 2 and Group 3 were given high fat diet. Stevia leaves were further added for six weeks in the diet of Group 3. **Results:** High fat diet induced NAFLD in rats was ameliorated significantly on treatment with stevia. On comparison of liver function tests in obese control with stevia treated group, the values of ALT and AST were significantly decreased ($p<0.05$). However, ALP and bilirubin were not decreased significantly. **Conclusion:** *Stevia rebaudiana* exerts hepatoprotective effect in restoring liver damage due to high fat diet induced NAFLD in Sprague Dawley rats.

Keywords: *Stevia rebaudiana*, NAFLD, liver damage, obesity, liver function tests

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INTRODUCTION

Prevalence of obesity has considerably increased over recent years all over the world. It is a systemic disorder that predisposes individuals to various complications and co-morbidities like hypertension, hyperlipidemia, diabetes mellitus, atherosclerosis and fatty liver disease. Obesity can also lead to liver problems just like alcohol misuse and drug abuse which can alter the liver enzymes. Non-Alcoholic Fatty Liver Disease (NAFLD) is due to collection of triglycerides in the hepatocytes which can be identified with radiological as well as histopathological findings like steatosis, lobular inflammation, hepatocyte ballooning and fibrosis. NAFLD can progress into Non-alcoholic Steatohepatitis (NASH), fibrosis and eventually into cirrhosis which can lead to hepatocellular carcinoma. NAFLD causes increased serum liver enzymes. There are various causes of fatty liver-induced hepatitis. Potential pathophysiology for fatty accumulation can be:

1) Reduced β-oxidation of fatty acids by mitochondria[1]
2) Increased endogenous synthesis of fatty acids[2]
3) Enhanced fatty acid transport to the liver[3]
4) Diminished metabolism of very low density lipoproteins (VLDL) or triglycerides[2].

There is still ongoing research to find out causes and effects of NAFLD. A few recent studies show high levels of hedgehog pathway activation in patients with advanced fatty liver disease. The suggestion still indicated that the hedgehog pathway is an adult liver repair regulator.[4] In NAFLD there is also progressive procoagulants imbalance from inflammatory steatosis to cirrhosis. The Liver Function Tests (LFTs) detect liver enzymes like alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) which are raised in the inflammation or damage of hepatocytes. Amongst different hepatic enzymes ALT has been associated with fatty liver disease which shows major role in metabolic syndrome. ALT is formed in liver cells and is also known as serum glutamic-pyruvic transaminase (SGPT). ALT is also used to evaluate the prognosis of liver disease.

To combat obesity, there has been a growing inclination towards substituting regular sugar with artificial sweeteners to decrease calorie consumption. However, the safety of artificial sweeteners has been a subject of debate since their discovery.[5] These substitutes have been associated with various health risks such as weight gain, potential carcinogenic effects, increased diabetes risk, and even reported links to depression.[6] Stevia, scientifically referred to as *Stevia rebaudiana* Bertoni, is a naturally occurring small perennial plant with sweet properties. Indigenous to the South American continent, Stevia is a commonly found shrub-like plant.[7] It belongs to the Asteraceae family of plants. Native people have been using the leaves of this plant since centuries in herbal teas for its sweet flavour and even used it in medicines. It was primarily introduced to other parts of the world by a Swiss botanist and naturalist named Moieses Santiago

Bertonie in year 1887 who learned the effects of Stevia from local Latin Americans.[8] Chemically the natural components of the stevia shrub is commonly known as stevioside or steviol glycosides which form the main characteristic sugary taste. In 1931 two French pharmacists isolated the steviols as steviosides extracts.[9] The main element constituting the sugary taste is stevioside present in stevia leaves. Japan was the foremost country to industrialize the commercial use of stevia in unpurified form as stevioside. Eventually the use of stevia and its extracts started commercially worldwide in health and food industries.[10] The effect of stevia as an anti-obesity, anti-diabetic and its help in controlling dyslipidemia has been reported.[11] The association of NAFLD with stevia treatment is still an ongoing process and very few investigations prove that stevia has effect on diminishing the NAFLD and there is requirement of further interventional researches to be carried out to explore the effects of stevia on NAFLD.[12]

The objective of this study was to see the effect of stevia leaves in restoration of some liver function tests in obese Sprague Dawley rats.

MATERIAL AND METHODS

This research experiment took place at the Physiology Department in collaboration with the Pathology Department of Islamabad Medical and Dental College, and National Institute of Health Islamabad after approval from College of Physicians and Surgeons Pakistan, over a duration of 18 months from Jul 2020 to Jan 2022. Ninety rats were selected for the study, and were divided into 3 groups of 30 healthy Sprague Dawley rats. The facilities provided for the animals complied with international standards.[13,14] The room was well ventilated and kept at controlled conditions of temperature (22–24 ºC). A 12-hour light and 12-hour dark cycle[9], was maintained in the animal house of NIH.[15] Animals were given water *ad libitum*.

Group 1 (n=30) was normal control on normal chow diet. Group 2 (Obese control, n=30) received a high fat diet with water during the entire study period. Group 3 (Obese on whole Stevia, n=30) was given dried, crushed Stevia leaves mixed with their food in a dose of 200 mg/Kg body weight, for a further period of 6 weeks adjusted weekly based on body weight.[16] At the end of the study period, blood sampling was done through intra cardiac puncture. The blood samples after clotting were centrifuged and serum was used to assess liver function tests (ALT, AST, ALP, and total bilirubin) using commercial kits on autoanalyzer (Selectra E fully automatic Chemistry Analyzer).

Statistical analysis was done on SPSS-24. The quantitative data like ALT, AST, ALP, and bilirubin were expressed as Mean±SD. One-way ANOVA and Post Hoc Tukey test were applied to see the differences between groups, and $p \leq 0.05$ was considered statistically significant.

RESULTS

One way ANOVA showed that there was statistically significant differences among the groups in ALT, AST, and Bilirubin ($p<0.05$), while ALP did not show significant differences ($p=0.063$) among the groups. (Table-1).

On comparison of liver function tests of normal group with obese control the differences between ALT, AST, and bilirubin were highly significant ($p<0.05$), while ALP was increased in obese control but this increase was not statistically significant ($p=0.163$). Comparison of normal group with stevia shows statistically significant differences in ALT only ($p<0.05$). On comparison of liver function tests in obese control with stevia group, the values of ALT and AST were significantly decreased ($p<0.0001$). The ALP and bilirubin were not decreased significantly ($p>0.05$) (Table-2).

Table-1: Comparison of serum ALT, AST, ALP, and Bilirubin among groups with one-way ANOVA

Parameter (Serum)	Normal control (n=30)	Obese control (n=30)	Stevia treated (n=30)	*p*
ALT(µ/L)	65.14±6.07	160.71±14.30	80.14±10.75	<0.001*
AST(µ/L)	53.86±6.09	111.57±23.94	61.00±6.66	<0.001*
ALP (µ/L)	302.71±31.7	332.0±18.0	333.85±31.17	0.063
Bilirubin (mg/dL)	0.79±0.16	1.00±0.12	0.91±0.11	<0.017*

*Significant

Table-2: *p*-Values for mean differences between groups on post-hoc Tukey's test

Groups Compared	Bilirubin	ALT	AST	ALP
Control *vs* Obese	0.027*	<0.001*	<0.001*	0.163
Control *vs* Stevia	0.287	0.046*	0.769	0.126
Obese *vs* Stevia	0.623	<0.001*	<0.001*	0.999

*Significant

DISCUSSION

Being overweight and obese is a major dilemma worldwide. Obesity is a major risk factor of cardiovascular disorders, metabolic derangements, carcinogenesis as well as musculoskeletal problems.[17] Non-alcoholic fatty liver disease (NAFLD) is a hallmark of obesity due to high calorie diet. Weight reduction is recommended to reverse the effects of NAFLD.[18] The most feasible way for obese people for caloric reduction is by adding non-nutritive sweeteners in the diet. Despite ongoing improvements in medicinal practices, safety of medicinal herbs and their effects is an active domain of research to treat fatty liver disease. As the novel researches prove that treating diseases with extracts are always more effective in treatment than herbs themselves.[19,20] Our effort was to find the effect of a herb *Stevia rebaudiana* on high fat diet inducted fatty liver in murine model. Liver is a sensitive organ and its

function can be assessed by measurement of raised liver enzymes, i.e., ALT, AST, ALP, and serum Bilirubin.[21] Our results demonstrated that there was a significant increase in liver enzymes in obese control rats which is consistent with recent work of Haung *et al*[22], who devised NAFLD model by inducing high fat diet to mice. They concluded that high fat diet can increase liver enzymes levels suggesting severe hepatocellular injury proved with their histological findings. Similar results were revealed in our study. The increase in level of liver enzymes in high fat diet induced rats is caused by formation of free radicals and protein glycosylation in the liver parenchyma.[23]

The ALT, AST, and even the bilirubin level were significantly decreased in our study to almost normal levels indicating hepatoprotective effect of the plant. The ALP and bilirubin that are markers of hepatobiliary tract and liver parenchyma, remained unaltered in stevia treated animals. Latha *et al*[24] compared protective effect of alcoholic extract of stevia leaves and stevioside in lipopolysaccharide induced liver injury of rats. They observed that these extracts of stevia significantly restored the raised ALT, and AST levels to normal. Our results of hepatic enzymes are consistent with Emam *et al*[25] who studied the effect of stevia in high fat diet induced diabetic rats and found that the parameters are significantly improved. AbdElwahab *et al*[26] showed no change in liver enzymes after stevia treatment compared with normal controls while aspartame caused significant elevation in ALT, AST, and even ALP. They concluded that altered hepatocellular function in metabolic syndrome reversed ALT by 62%, AST by 57% and ALP by 41% with administration of stevia leaves that was far better than results of aspartame. Our findings of significant restoration of liver enzymes are in disagreement to them. A possible reason for that may be their use of commercial alcohol treated extract of rebiana, while we used whole stevia leaves; and they made hepatotoxic model with alloxan while our rat model was high fat induced NAFLD.

Our results are controversial to similar study done by Ranjbar *et al*[27]. They studied the effects of stevial extract in high fat diet induced metabolic syndrome rats. They concluded that on administration of stevial extract the hepatic impairment deteriorates by increased ALT but no change in AST and ALP. Their results may be different from ours because they used different non-organic extracts of stevia and a commercially extracted rebiana with high extraction and purification quality[28], while we used dried whole leaves. Ranjbar *et al* also concluded that their regime dosage differences did not affect the amelioration of high fat liver changes.[27] The effect of different doses of stevia extract on LFTs was also compared by Elanga *et al*[29] by giving 25, 250, 500 and 100 mg/Kg/day dosage to female rats in groups. Their treatment with stevia extract showed comparable declension effects of liver enzymes by 500 and 1,000 mg dosage, while we used just 250 mg/Kg regime and got similar hepatoprotective effects. Our results proved that using whole leaf causes same reversal of not only ALT, and AST, but also ALP.

CONCLUSION

Stevia has hepatoprotective effect in high fat diet induced NAFLD which is exhibited by restoration of liver enzymes.

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Address for Correspondence:

Dr Sadia Mubarak, Department of Physiology, Islamabad Medical and Dental College, Islamabad, Pakistan. **Cell:** +92-332-5020560

Email: sadia.smcian@gmail.com

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SL: Acquisition and analysis of data
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ORIGINAL ARTICLE

EFFECTS OF L-CARNITINE THERAPY ON FAT AND GLUCOSE METABOLISM AMONG MELDONIUM INDUCED CARNITINE DEPLETION IN ALBINO WISTAR RATS

Sumayya Qazi, Mumtaz Ali Qureshi, Abroo Fatima Qazi*, Amin Fahim, Ghulam Shah Nizamani**, Bakhtawar Qureshi*****

Department of Biochemistry, *Physiology, **Pathology, ***Community Medicine, Isra University, Hyderabad, Pakistan

Background: L-carnitine therapy has a potential role in lipid metabolism and glucose homeostasis. Despite its promising effects, further investigation is warranted. This study aimed to determine the impact of L-carnitine therapy of low and high doses on fat and glucose metabolism of albino Wistar rats after Meldonium induced carnitine depletion. **Methods:** A total number of 48 albino Wistar rats were recruited and divided into four groups 12 rats in each group. The bedding material was changed every second day and all animals were given Kaytee Supreme Fortified Daily Diet rat food and clean distal water *ad libitum* at room temperature. The experiment was commenced after 10 days of acclimatization. **Results:** Both high (300 mg/Kg) and low-dose (500 mg/Kg) L-carnitine significantly improved (p<0.05) blood glucose levels, TC, LDL, and HDL after four weeks of L-carnitine therapy. However, high-dose therapy showed significantly higher HDL improvements and decreased TC and LDL levels than low-dose therapy (p<0.05) among Meldonium carnitine-depleted rats. **Conclusion:** L-carnitine high dose therapy (500 mg/Kg of body weight) had a more potent effect on lipid profile in comparison to low dose (300 mg/Kg of body weight).

Keywords: Blood glucose, Carnitine, Cholesterol, lipoproteins, HDL, LDL

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INTRODUCTION

L-carnitine (LC) is a quaternary amine (3-hydroxy-4-N-trimethylaminobutyrate) that is mainly responsible for the transportation of long-chain fatty acid across the inner membrane of mitochondria for β-oxidation and adenosine triphosphate (ATP) synthesis.[1,2] Besides that, L-carnitine also prevents the pooling of acetyl-CoA that is generated during the process of β-oxidation, thereby preventing the accumulation of fatty acids, protecting the cellular membrane, controlling glycogenesis and ketogenesis and toxic metabolites elimination.[3,4] Evidence is also found in the literature that L-carnitine's effects have improved insulin sensitivity and glucose tolerance.[5,6] Various mechanisms have been put forward in which the desirable impact of L-carnitine on glucose metabolism is established in the literature that includes: 1) mitochondrial oxidation of long-chain acyl-CoA as its accumulation causes insulin resistance 2) improving acety L-CoA to CoA ratio that is essential for pyruvate dehydrogenase complex (PDHC) activity 3) increasing the expression of essential enzymes responsible for gluconeogenesis and glycolysis 4) increasing insulin signalling cascade by improving associated related gene expression and 5) improving insulin growth factor (IGF-1) axis and IGF-1.[7–9] Hence, in this way, L-carnitine is considered an essential factor in regulating glucose metabolism and improving insulin resistance. Multiple studies are also available on data search in which the therapeutic effects of L-carnitine drugs have been discussed. The authors have concluded that various metabolic disorders and carnitine deficiency can effectively be treated by administering exogenous carnitine as a mode of treatment.[10,11] Extensive research has been conducted in recent times in which the positive role of L-carnitine therapy on conditions like chronic kidney disease[12], male and female fertility[13], premature neonates[14], pregnancy[15], fatty liver diseases[16] and myalgic encephalomyelitis[17] has been performed. However, literature still recommends further evidence, particularly its role in metabolic conditions like hyperglycaemia and high blood cholesterol levels. In a study that was performed on mice to whom the non-alcoholic fatty liver disease has been induced through high-fat diet (HFD), the effects of L-carnitine versus nicotinamide adenine nucleotide (NAD^+) versus combination therapy including both LC+NAD and the authors have observed that combination therapy produced better results in improving glucose and fat metabolism in comparison to LC and NAD+ therapy alone.[18] In another study that has been a systematic review and meta-analysis performed on 16 trial studies, it has been concluded by the authors that L-carnitine has a potential role as an adjunctive therapy in diabetes; however, further researches are required for more potent evidence.[19] In order to elucidate the role of L-carnitine in glucose metabolism and insulin resistance, this study aims to determine the effect of effects of low and high L-carnitine therapy doses on fat and glucose metabolism in Wistar rats after Meldonium-induced L-carnitine depletion.

METHODOLOGY

The Comparative Experimental study was conducted in the Department of Biochemistry in collaboration with the Diagnostic and Research Laboratory of Isra University Hospital, Hyderabad from January 2019 to August 2019.

The Animals weighing 200–250 gm were taken and kept at the animal house of Agricultural University, Tando Jam. The animals were housed in polypropylene cages of 43×27×15 cm. Laboratory conditions were maintained at a temperature of 20–25 °C, and they were exposed to 12 hours of light and 12 hours of dark cycle. The bedding material was changed every second day, and all animals were given Kaytee Supreme Fortified Daily Diet rat food and clean distal water ad libitum at room temperature. The experiment was commenced after ten days, and the animals were kept in the lab environment for acclimatization.

A total number of n=48 albino Wistar rats were recruited and divided into four groups, 12 rats in each group. Group A was kept under control, whereas animals in the remaining three groups were administered meldonium mixed with food in the form of powder. Meldonium was administered 100 mg/Kg of body weight for L-carnitine depletion for ten days. No treatment was given after meldonium induction to animals in group B. In contrast, animals in groups C and D were given L-carnitine at a low dose of 300 mg/Kg of body weight and LC, a high dose of 500 mg/Kg daily as a therapeutic agent for four weeks. Blood samples were collected twice through the tail prick method after cleaning it with an alcohol swab at baseline and after four weeks of treatment. A baseline measurement was taken after meldonium induction; post values were measured after four weeks of LC treatment; blood samples were taken after 5 hours of fasting.

Collected blood samples were analyzed for the levels of glucose by using an Accu-Check active glucose meter kit, and levels of cholesterol, including total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL), were measured using a Cholesterol ELISA assay kit ab285242 (BioVision an Abcam Company).

RESULTS

A total number of n=48 rats were divided into four groups. Each group contain n=12 rats. The weight of rats in each group was measured and compared with another group to maintain the homogeneity of samples between groups. The description is provided in Table-1.

After ten days of keeping animals in the laboratory for acclimatization, meldonium at 100 mg/Kg of body weight was given by mixing with food in the form of powder for ten days (except on animals in the control group) and afterwards, blood test was performed to record the values of fasting blood glucose and lipids levels (TC, HDL and LDL) at baseline followed by therapeutic administration of LC at low dose (300 mg/Kg of body weight) and high dose (500 mg/Kg of body weight) for four weeks to animals in group C and D after which final blood readings were taken to compare the levels of fasting blood sugar (FBS) and lipids from baseline.

Analysis of the findings revealed that both low and high doses of L-carnitine therapeutic drugs were effective in improving fasting blood sugar levels after four weeks of treatment. The values of blood glucose after inducing carnitine depletion through meldonium reduced to 75.6±4.8 mg/dL in group B, 76.3±2.7 mg/dL in low dose carnitine group (group C) and 76.28±2.5 mg/dL in high dose LC group (group D) that had remained non-significant altered I=0.025 in group B with a mean of 76.6±4.9 mg/dL, improved significantly $p<0.05$ in low dose LC group with a mean of 85.62±2.98 mg/dL and in high dose LC group the mean value of 86.1±5.1 mg/dL was found that was significantly different $p<0.001$ from baseline readings (Table-2).

Further, to determine efficacy between low and high-dose LC therapy groups, t-tests were performed that revealed a significant mean difference $p<0.05$ between the control, medium and low and high-dose LC therapy groups. In contrast, no significant ($p=0.16$) mean difference between low and high-dose LC therapy group was found on FBS levels (Table-3).

Further lipid profile analysis was performed at baseline and after four weeks of treatment. Analysis revealed a significant mean difference at $p<0.05$ in the TC, LDL and HDL levels in both low and high doses of L-carnitine treatment (Table-4).

Further, between-group analysis determined the difference between low-dose and high-dose carnitine treatments. Results revealed that high-dose carnitine therapy was significantly effective at $p\leq0.05$ in improving HDL levels and reducing TC and LDL levels compared to low-dose L-carnitine therapy (Table 5).

Table-1: Mean weight of animals allocated in groups

Variables	Number of Samples	Mean±SD	*F*	*F*-critical	*p*
(Group A) Control	12	218.5±5.8	0.619	2.816	0.606
Group B	12	219.9±6.57			
Group C	12	220.9±6.8			
Group D	12	217.8±5.15			

Table-2: Within group analysis of FBS levels mg/dL

Variables	Baseline±SD	Post±SD	*t*-stat	*P*
(Group A) Control	95.8±2.7	96.1±2.1	-0.34	0.36
Group B	75.6±4.8	76.6±4.9	0.68	0.25
Group C	76.3±2.7	85.62±2.98	16.04	0.0001
Group D	76.28±2.5	86.1±5.1	11.72	0.0001

Group A=Control; Group B=Meldonium; Group C=Meldonium+LC 300 mg/Kg; Group D=Meldonium+LC 500 mg/Kg

Table-3: Within group analysis of FBS levels mg/dL

Variables	Groups	Mean±SD	F-ratio	F-critical	*p*	Factors Groups	*p*
Control Group A	B	76.6±4.9	499.07	2.814	0.0001	B	<0.001
	C	85.62±2.98				C	<0.001
	D	86.1±5.1				D	<0.001
Group B	A	96.1±2.1				A	<0.001
	C	85.62±2.98				C	<0.001
	D	86.1±5.1				D	<0.001
Group C	A	96.1±2.1				A	<0.001
	B	76.6±4.9				B	<0.001
	D	86.1±5.1				D	0.16
Group D	A	96.1±2.1				A	<0.001
	B	76.6±4.9				B	<0.001
	C	85.62±2.98				C	0.16

Group A=Control; Group B=Meldonium; Group C=Meldonium+LC 300 mg/Kg; Group D=Meldonium+LC 500 mg/Kg

Table-4: Lipid profile values at baseline and after four weeks of LC treatment

Variables	Group A	Group B	Group C	Group D
TC mg/dL				
Baseline	134.91±13.41	252.55±6.39	252.36±3.47	252.09±11.44
Post	134.36±12.84	252.82±5.42	202.09±7.5	190.45±4.61
***t*-stat**	0.76	-0.31	18.59	16.98
***t*-critical**	1.81	1.81	1.81	1.81
p	0.23	0.37	0.001	0.001
LDL mg/dL				
Baseline	123.27±4.45	161.18±4.69	161.09±2.98	160.36±4.11
Post	123.18±4.98	159.55±2.42	139.18±5.55	129.64±4.39
***t*-stat**	0.09	1.61	14.53	15.33
***t*-critical**	1.81	1.81	1.81	1.81
p	0.46	0.06	0.001	0.001
HDL mg/dL				
Baseline	57.82±2.71	30.09±14.19	33.91±3.36	32.82±3.84
Post	58.27±2.69	31.36±10.05	42.18±2.48	57±4.36
***t*-stat**	-1.11	-0.95	-7.07	-11.48
***t*-critical**	1.81	1.81	1.81	1.81
p	0.29	0.18	0.001	0.001

Group A=Control; Group B=Meldonium; Group C=Meldonium+LC 300 mg/Kg; Group D=Meldonium+LC 500 mg/Kg

Table-5: Comparing effectiveness of low dose and high dose LC therapy on lipid profile

Variables	Group C	Group D
TC (mg/dL) at week four	202.09±7.5	190.45±4.61
***t*-stat**	5.93	
***t*-critical**	1.81	
p	0.0001	
LDL (mg/dL) at week four	139.18±5.55	129.64±4.39
***t*-stat**	3.61	
***t*-critical**	1.81	
p	0.002	
HDL (mg/dL) at week four	42.18±2.48	57±4.36
***t*-stat**	-13.69	
***t*-critical**	1.81	
p	0.0001	

DISCUSSION

The findings of this study revealed that L-carnitine low dose and high dose treatment, i.e., 300 mg/Kg and 500 mg/Kg of body weight respectively, was found to be equally effective in improving the fasting blood sugar levels among meldonium-induced carnitine depletion rats. However, on lipid profile levels that include TC, HDL and LDL, high-dose carnitine treatment was found to be significantly $p<0.05$ more effective in increasing HDL levels and reducing TC and LDL levels compared to the dose treatment approach. The findings of this study were according to the findings of another conducted study in which L-carnitine supplementation therapy of 50 mg/Kg of body weight recommended to children diagnosed with chronic kidney disease (CKD) and were going through haemodialysis turned out to be significantly effective ($p<0.05$) in improving the levels of c-reactive protein and fasting blood sugar levels.[20]

In another study, an association between L-carnitine levels and various cardiovascular disease biomarkers was identified, and it concluded that a significant ($p=0.042$) negative correlation exists between L-carnitine and triacylglycerol levels and blood glucose levels ($p=0.048$). In contrast, a significant positive correlation ($p=0.049$) exists between LC and HDL levels.[21] The findings of that study reflect our findings that LC treatment effectively maintained blood glucose levels and controlled cholesterol and LDL levels. Besides that, it also had a crucial impact on increasing HDL levels in blood.

A study was conducted to determine the effects of three different doses of L-carnitine that were 50, 100, 200 and 300 mg/Kg of body weight, being given to streptozotocin (STZ) induced diabetic rats to determine its impact on the histopathology of the pancreas of STZ induced diabetic rats and compared the same with diabetic control group it was found that LC of 300 mg/Kg of body weight found to be better than other low dose LC treatment and concluded that LC treatment approach had a significant role in improving histopathology of the pancreas and had an antioxidant action as well.[22]

Thus, based on the literature, it was evident that LC treatment significantly improved blood sugar, TC and LDL levels and increased HDL levels. However, for more robust findings based on its effectiveness in response to different dosages, further studies are required to gather more conclusive evidence.

CONCLUSION

The study has provided evidence that L-carnitine treatment decreased fasting blood sugar levels, total cholesterol, and LDL levels, as well as improved HDL levels among meldonium-induced carnitine depletion rats. L-carnitine high-dose therapy (500 mg/Kg of body weight) had a more pronounced effect on lipid profile than low-dose therapy (300 mg/Kg). The impact of L-carnitine on blood glucose levels in both high and low-dose treatment approaches is found to be similar.

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Address for Correspondence:

Dr Sumayya Qazi, Department of Biochemistry, Isra University, Hyderabad, Pakistan. **Cell:** +92-334-2166554
Email: drsumayyaqazi@gmail.com

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MAQ: Research concept and design, Critical revision of article and final approval of article
AFQ: Critical revision of article and final approval
AF: Data analysis and interpretation and writing of article
GSN: Collection and assembly of data, Critical revision of article
BQ: Collection and assembly of data, Critical revision of article

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ORIGINAL ARTICLE

OUTCOME OF EARLY INITIATION OF POST-SURGERY FEEDING AFTER COLOSTOMY REVERSAL IN CHILDREN

Muhammad Siddique, Tariq Iqbal*, Fawad Mueen Arbi*, Fayaz Ahmad*, Tabinda Yasmin, Ammar Bin Saad*****

Department of Paediatrics Surgery, *Surgery, Bahawal Victoria Hospital, **Pathology, Sadiq Abbasi Hospital, Bahawalpur, ***Ayub Medical College, Abbottabad, Pakistan

Background: Children undergoing colostomy reversal surgery often face uncertainties regarding the optimal timing for reintroducing feeding postoperatively. Early initiation of post-surgery feeding is of interest due to its potential to hasten recovery and reduce hospital stay. The impact of early feeding initiation is unclear. Objective of this study was to find out the outcome in terms of hospital stay and post-surgery complications of early initiation of post-surgery feeding after elective colostomy reversal in children. **Methods:** From 6 Jun 2022 to 10 Oct 2023, a non-randomized controlled trial was conducted at Bahawalpur, analyzing 93 children under 15 years with status colostomy following elective reversal. Patients were allocated to Group-A (traditional oral feeding) or Group-B (early oral feeding) at the surgeon's discretion, with outcomes including post-surgery hospital stay duration and complications. **Results:** Out of a total of 93 patients, 49 (52.7%) were boys and 44 (47.3%) girls. Overall, mean age was 4.86±3.27 years. Anastomotic shock was noted among 6 (6.5%) patients while 9 (9.7%) patients had superficial surgical site infection. None of the patients needed repeat surgery for any post-surgery complications. No significant difference in terms of anastomotic leak (p=0.3983), and superficial surgical site infection (p=0.4980) were observed. Significantly shorter duration of post-surgery hospital stay was recorded among patients of Group-B when to compared to Groups-A (6.49±1.14 *vs* 8.90±1.27, p<0.0001). **Conclusion:** Early initiation of enteral feeding post elective colostomy reversal in children resulted in significantly reduced duration of post-surgery hospital stay.

Keywords: Colostomy, complications, outcome, oral feeding

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INTRODUCTION

In patients having elective colostomy reversal, bowel rest is commonly preferred adopting nasogastric (NG) decompression and subsequent wait till bowel movement turns normal allowing passage of flatus/faeces on post-surgery day 3 or more.[1,2] Till that time patients are not allowed to have enteral feeding while NG tube is kept *in situ* until the content is reduced and turned clear. There are many causes of anastomosis leakage and it is often presumed that fasting protects the anastomosis from any possible complications allowing hermetic closure of anastomosis prior to the start of enteral feeding.[3,4]

In the recent past, some researchers have challenged these traditional approaches as not much scientific evidence exists about these ideas while it is also true that effects of prolonged fasting among children undergoing elective colostomy reversal has not yet been evaluated properly.[5] Some researchers have pointed out resumption of early feeding on post-surgery day 1 as it seems functionally possible.[6] Even in the absence of oral feeding, around 1.5 to 2 litres of gastrointestinal and pancreatic sections enter and pass through the distal bowel and are absorbed.[7]

Following surgery, return to bowel function and motility usually happens between 6 to 12 hours in small bowel, 12 to 24 hours in stomach while large bowel usually takes 48 to 72 hours.[8] Dag A *et al* analyzing early versus traditional oral feeding among patients following colostomy reversal found early feeding to be beneficial.[9] Lewis SJ *et al* in their systemic review and meta-analysis found feeding within 24 hours following laparotomy to be well tolerated.[10]

GI blood flow is reduced in cases following different kinds of critical medical conditions while manipulations in the intestine might initiate pathogenesis of intestinal oedema that can alter GI blood blow and enhance inflammatory response through activation of macrophages and invasion of neutrophils.[11] Early oral feeding may improve the nutritional status and help improvement in wound healing which in turn can reduce post-surgery complications. Some international data exists regarding use of early feeding in patients following colostomy reversal but no local study exists. Majority of the studies conducted in the past have been retrospective analyses which have its own limitations.

The present study was aimed to find out outcome in terms of post-surgery complications of early initiation of post-surgery feeding after elective colostomy reversal, and duration of hospital stay in children.

MATERIAL AND METHODS

This non-randomized controlled trial was conducted at the Department of Paediatric Surgery, Bahawal Victoria Hospital, Quaid-e-Azam Medical College, Bahawalpur, from 6 Jun 2022 to 10 Oct 2023 after approval from

Institutional Ethical Committee. Written consent was sought from parents/guardians of all study participants.

A total of 93 children aged up to 15 years were enrolled. Indications for elective colostomy were peritonitis following enteric fever, anorectal malformation, or rectal atresia (as per medical history, clinical examination, radiological and laboratory investigations). All patients had elective colostomy after adequate proximal mechanical bowel washing with polyethylene glycol and non-residue diet for 2 days while clear water and oral rehydration salt for 1-day prior to surgery with distal mechanical bowel wash using normal saline till the visibility of clear effluent. Children having seizures, cardiovascular defects, complications of past surgeries, more than 2 past abdominal surgeries, adhesions, or those with gross luminal disparity between proximal and distal colon during surgery were excluded.

Demographic data were recorded and patients were non-randomly distributed into 2 groups. A total of 93 patients underwent colostomy reversal. Group-A had 63 patients who were kept *nil-per-os* until documentation of bowel functioning showing passage of flatus or faeces, normally on 3rd post-surgery day. The NG tube was kept *in situ* until the content reduced and turned clear. Group-B had 30 patients who started enteral diet the next morning within 16 to 24 hours of 1st post-surgery day. Feeding was initiated with clear water which was followed by breast milk and other liquid diet after 3–4 hours. The NG tube was removed in the morning of the 1st post-surgery day. Reinsertion of NG tube was done if a patient had 2 episodes of vomiting, having more than 100 mL within 24 hours in absence of bowel movements.

All patients were observed for post-surgery complications like anastomotic leak or superficial surgical site infections which were handled as per institutional protocols. Patients were discharged when they did not have any post-surgery complication and had established full-enteral feeding. Duration of post-surgery hospital stay and post-surgery complications were noted among all patients.

Data were recorded on a special proforma, and analysed using SPSS-26. Qualitative variables like gender, area of residence and post-surgery complications were presented as frequency and percentages. Quantitative variables like age and duration of hospital stay were shown as Mean±SD. Chi-square test was applied to compare qualitative variables and independent sample *t*-test was used to compare quantitative variables between study groups considering $p \leq 0.05$ as statistically significant.

RESULTS

A total of 93 patients fulfilled the inclusion and exclusion criteria. Out of the total, 49 (52.7%) were boys and 44 (47.3%) were girls. Overall, mean age was 4.86±3.27 years (Range: 1–15 years). There were 50 (53.8%) patients from rural areas 43 (46.2%) were from urban areas (Table-1).

Anastomotic shock was noted in 6 (6.5%) patients while 9 (9.7%) patients had superficial surgical site infection. None of the patients needed repeat surgery for post-surgery complications and were managed conservatively (Table-2).

Overall, mean duration of post-surgery hospital stay was 7.92±1.38 days (Range: 4–14 days). Significantly shorter duration of post-surgery hospital stay was recorded among patients of Group-B compared to Groups-A (6.49±1.14 *vs* 8.90±1.27, $p<0.0001$) (Table-3). Following post-surgery initiation of the feeding, none of the patients needed reinsertion of NG tube. No mortality was reported in the present study.

Table-1: Characteristics of patients between both study groups (n=93)

Characteristics		Group-A (n=63)	Group-B (n=30)	*p*
Gender	Boys	32 (50.8%)	17 (56.7%)	0.5959
	Girls	31 (49.2%)	13 (43.3%)	
Age in Years (Mean±SD)		4.96±3.18	4.72±3.48	0.7422
Area of Residence	Rural	35 (55.6%)	15 (50.0%)	0.6154
	Urban	28 (44.4%)	15 (50.0%)	

Table-2: Distribution of post-surgery complications between both study groups (n=93)

Post-Surgery Complications	Group-A (n=63)	Group-B (n=30)	*p*
Anastomotic leak	5 (7.9%)	1 (3.3%)	0.3983
Superficial surgical site infection	7 (11.1%)	2 (6.7%)	0.4980

Table-3: Comparison of mean duration of post-surgery stay between both study groups (n=93)

Mean duration of post-surgery stay (days)	Group-A (n=63)	Group-B (n=30)	*p*
	8.90±1.27	6.49±1.14	<0.0001

DISCUSSION

Mucosal epithelium of the bowel is thought to completely seal following first 24 hours of post-surgery period.[3] Studies conducted in animals have found early feeding to accelerate wound and anastomosis healing.[12] Early feeding is thought to reserve mucosal atrophy induced by the starvation while increasing the anastomotic collagen deposition and strength.[13] Early feeding is known to delay post-surgery ileus helping wound healing and reducing sepsis.[14] Post-surgery ileus is described as a significant reason for patients being kept nothing *per os* in the post-surgery period. To assess post-surgery ileus, the first NG tube is removed on the morning of postoperative day 1, and subsequent monitoring is conducted closely. In the present study, following post-surgery initiation of the feeding, none of the patients needed reinsertion of NG tube. Post-surgery nutrition is considered to be an important factor influencing post-surgery complications and duration of

post-surgery hospital stay.[11] None of our patients received complete parenteral nutrition during the initial post-surgery period.

Although no statistically significant differences were observed in terms of post-surgery complications between both groups, patients in early feeding group had lesser rate of post-surgery complications. Ghosh A *et al*[15] found significantly low rates of post-surgery complication among children in early feeding group when compared to controls. Some researchers[16] have shown that early feeding might affect healing and may cause anastomotic leakage, but this was not evident in present study as 3.3% in early feeding group had anastomotic leak *vs* 7.9% in traditional feeding group.

We noted significantly low post-surgery duration of hospital stay among patients of early feeding group when compared to those in traditional feeding group. Duration of hospital stay is regarded as one of the major factor influencing patients' satisfaction with the treatment process. Nematihonar B *et al*[17] analysing early *vs* delayed post-surgery oral feeding among patient undergoing colostomy reversal found patients in early feeding group to have higher overall satisfaction in terms of treatment process as per visual analogue scale when compared to delayed post-surgery oral feeding. Paul SK *et al*[2] comparing early versus traditional oral feeding following colostomy closure in children found significantly less post-surgery duration of hospital stay in early oral feeding group which is quite similar to our study. Gosh A *et al*[15] also found post-surgery duration of hospital in early feeding groups among patients undergoing colostomy reversal to be significantly less when compared to traditional feeding group.

CONCLUSION

In children following elective colostomy reversal, early initiation of enteral feeding was found having statistically better outcome in terms of duration of post-surgery hospital stay.

LIMITATIONS AND RECOMMENDATIONS

Being a single centre study, conducted on a relatively small sample size with non-randomized sample allocation were some of the limitations of this study. Large scale studies will further verify our findings.

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Address for Correspondence:

Dr Fawad Mueen Arbi, Department of Surgery, BV Hospital, Bahawalpur, Pakistan. **Cell:** +92-300-7809970
Email: fawad.arbi@gmail.com

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ORIGINAL ARTICLE

FREQUENCY OF TYPE-II RESPIRATORY FAILURE IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Jalal Dildar, Hamza Munir*, Amanullah, Asim Khan***, Fauzia Aitazaz†, Mohsin Ali††**

Institute of Kidney Diseases, *Department of Community Medicine, Muhammad College of Medicine Peshawar, **Department of Physiology, Saidu Medical College, Swat, ***Department of Medicine, Khalifa Gul Nawaz Teaching Hospital, Bannu, †Department of Physiology, AJK Medical College, Muzaffarabad, Pakistan, ††PhD Scholar Department of Pharmaceutical Sciences, Ghent University, Belgium

Background: In chronic obstructive pulmonary disease (COPD) patients the alveoli do not ventilate fully due to bronchial obstruction. This leads to the incomplete ventilation, incomplete clearance of carbon dioxide causing hypercapnia. There are some muscular abnormalities also in COPD patients. All these pathogeneses lead to type 2 (hypercapnic) respiratory failure. The aim of this study was to determine the frequency of type II respiratory failure in patients with COPD. **Methods:** This descriptive cross-sectional study was conducted at Department of Medicine, Hayatabad Medical Complex, Peshawar from 21st Aug 2021 to 20th Feb 2022. A total of 129 patients with COPD were enrolled. COPD was diagnosed based on clinical findings and confirmation with spirometry showing FEV_1/FVC less than 70% of predicted. Confirmation of type II respiratory failure was done by Arterial-blood gas (ABG) test on heparinized arterial blood sample analysis showing hypercapnia (Pa_{CO_2} ≥45 mmHg). **Results:** Type II respiratory failure was observed in 31 patients (24%); 19 (22.9%) patients with type respiratory failure had age more than 55 years. Eight (36.4%) patients with type II respiratory failure had GOLD stage 4 COPD. Age of the patients ranged from 40 to 65 years with mean age 53.410±9.362 years. Male to female ratio was 2.4:1. **Conclusion:** Type 2 respiratory failure is more prevalent in elder patients above 55 years of age, and is more common among males. The severity of COPD does not appear to have a direct relationship with the occurrence of type 2 respiratory failure.

Keywords: COPD, Type II Respiratory Failure, Arterial Blood Gases, Hypercapnia

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INTRODUCTION

The Global Initiative for Chronic Obstructive Lung Disease (GOLD) was created in 2001 and defines COPD as 'a disease state characterized by airflow limitation that is not fully reversible.[1] The airflow limitation is usually both progressive and associated with an abnormal inflammatory response of the lungs to noxious particles or gases'. COPD comprises a diverse group of clinical syndromes that share the common feature of limitation of expiratory airflow.[2] The American Thoracic Society defines COPD in terms of chronic bronchitis and emphysema. COPD can also be classified with respect to both phenotype and disease severity. It is a heterogeneous disease process that varies greatly from person to person with respect to lung pathology, natural history of disease, and co-morbidity. The result of this heterogeneity is that different researchers have championed alternative hypotheses about COPD development over the past four decades: The British hypothesis stated that the presence of cough and sputum was the key factor in COPD, and the Dutch hypothesis pointed to the presence of increased airways responsiveness.[3,4] Less widely known hypotheses stressed the part of genetic factors (the Swedish hypothesis) and the role of impaired repair processes in the development of emphysema (the American hypothesis). All these hypotheses probably have elements of truth since COPD is a classic gene-by-environment disease with various manifestations that include increased airways reactivity, a characteristic response to infections, abnormal cellular repair, and development of complications or co-morbid disorders.[5] An interesting genetic area for COPD susceptibility is chromosomal band *15q25*.[1] The cholinergic receptor, nicotinic, alpha 3 (neuronal) gene, *CHRNA3*, the cholinergic receptor, nicotinic, alpha 5 (neuronal) gene, *CHRNA5*, and the iron-responsive element binding protein 2 gene, *IREB2*, are among the genes in this region that have been linked to a probable role in COPD. Both *CHRNA3/5* and *IREB2* have been recognized as viable candidates for a role in COPD susceptibility and progression by GWAS and integrative genomics techniques. It's interesting to note that these genes appear to play very diverse roles in the pathophysiology of COPD, despite being adjacent to each other genomically.[6]

Patients of COPD have been estimated to be 174.5 million only in 2015 which include both moderate to severe chronic obstructive pulmonary disease which have increased by 44.2% from the value in 1990.[7] It is estimated that up to 2040 COPD is expected to rise from 9th to 4th leading cause of life-years lost. Only in 2015 about 3.2 million people died of COPD globally (an increase of 11.6% compared with 1990).[8] The COPD patients frequently present with acute exacerbation. In COPD patients the alveoli do not ventilate fully due to bronchial obstruction. This leads to the incomplete

ventilation, incomplete clearance of CO_2 causing hypercapnia. There are some muscular abnormalities also in COPD patients. All these pathogeneses lead to type 2 (hypercapnic) respiratory failure.[9] In addition to other treatments home base management like home oxygen therapy are also indicated for management of COPD.[10] In a study 231 COPD patients were enrolled in which hypercapnic respiratory failure (Pa_{CO_2} ≥45 mmHg) was present in 58 (25%) patients of which, 20 (9%) had Pa_{CO_2} ≥50 mmHg. That study further evaluated that hypercapnia was more in the higher GOLD stage. In that study 26 (15%) patients of hypercapnic respiratory failure (Pa_{CO_2} >45 mmHg) were in need of day and night oxygen therapy.[11] Another study showed that hypercapnic respiratory failure (Pa_{CO_2} ≥45 mmHg) was present in 65 (28.1%) patients and 23 (10%) had Pa_{CO_2} ≥50 mmHg. Increased BMI, decreased forced vital capacity, and increased HCO_3^- level were significant independent predictors of hypercapnia. The overall mortality was 19.5% in patients with COPD and hypercapnia.[11]

The aim of current study was to determine the frequency of type 2 respiratory failure (hypercapnic) in patient presenting with chronic obstructive pulmonary disease at tertiary care hospital in Peshawar.

MATERIAL AND METHODS

This descriptive cross-sectional study was conducted from 21st Aug 2021 to 20th Feb 2022 at the Department of Medicine, Hayatabad Medical Complex, Peshawar, after approval from Ethical Committee of the Hospital. Patients admitted from the Out-patients Department were evaluated. Sample size was calculated using WHO sample size formula using the proportion (expected frequency of type 2 respiratory failure, p=25%[5], margin of error of 7.5% and with confidence interval of 95%. Non-probability consecutive sampling technique was applied. All known COPD patients aged 40–65 years with symptoms of shortness of breath (respiratory rate >22 breaths/minutes) referred to Medical Department were included. Patients with a documented history of physical abnormality of chest, Glasgow comma scale (GCS) lower than 5 and patients having lung pathology other than COPD were excluded from the study.

Written informed consent was taken from all participants of the study. All patients were managed as per hospital's protocol. Age, sex, duration of COPD, weight, height, and BMI were recorded. Spirometry was done using Contec® SP100 machine and the patient were classified as per GOLD stage. Patients' arterial blood samples were taken in a heparinized syringe under aseptic conditions and were sent for Arterial Blood Gases (ABGs) analysis and Pa_{CO_2} was recorded. Type 2 respiratory failure was labelled when Pa_{CO_2} was ≥45 mmHg.

Data was collected on a designated proforma, and analysed on SPSS-22. Mean±SD was calculated for continuous variables like age, weight, height, BMI, duration of COPD, predicted FEV_1, and Pa_{CO_2} level. Categorical variables like gender, type 2 respiratory failure and Gold stages were presented with frequency and percentages. Effect modifiers like age, sex, duration of COPD, GOLD stage, BMI were stratified against the type 2 respiratory failure. Post stratification Chi-square test was applied and p<0.05 was taken significant.

RESULTS

A total of 129 patients were recruited comprising of 90 (70.5%) male and 38 (29.5%) female patients. The age of the patients ranged from 40 to 65 years. Patients' demographics are shown in Table-1. The classification of the patients according to GOLD severity is shown in Table-2. Majority of the patients had stage-2 (moderate) COPD. It was found that 31 (24%) patients had type-II respiratory failure. Stratification of type 2 respiratory failure with respect to gender, BMI and GOLD stage are shown in Table-3.

Table-1: Patients demographics (Mean±SD) (n=129)

Demographics	Mean±SD
Age (Years)	53.41±9.362
Weight (Kg)	75.54±11.342
Height (Cm)	171.44±9.270
BMI (Kg/m^2)	25.78±4.08789
Disease Duration (months)	18.89±10.006
FEV_1 Predicted (%)	59.42±11.66

Table-2: Frequency and percentage of patients according to GOLD standards (n=129)

GOLD Stage	Frequency	Percent
Stage 1 (mild)	17	13.2
Stage 2 (moderate)	59	45.7
Stage 3 (severe)	31	24.0
Stage 4 (very severe)	22	17.1

Table-3: Stratification of COPD [n (%)]

Parameter	Yes	No	Total	p
Gender				
Male	25 (27.5)	66 (72.5)	91	0.031
Female	6 (4.6)	32 (95.4)	38	
BMI				
Healthy (BMI=18.5–5 Kg/m^2)	6 (9.2)	59 (90.8)	65	0.752
Overweight (BMI=25.1–30 Kg/m^2)	17 (41.5)	24 (58.5)	41	
Obese (BMI= >30 Kg/m^2)	8 (34.8)	15 (65.2)	23	
GOLD Severity				
Mild	2 (11.8)	15 (88.2)	17	0.817
Moderate	13(22.0)	46 (78.0)	59	
Severe	8 (25.8)	23 (74.2)	31	
Very Severe	8 (36.4)	14 (63.6)	22	

DISCUSSION

This study aimed to explore the frequency of type II respiratory failure in COPD. By analyzing the collected data and findings, we strive to gain insights into the impact of COPD on respiratory function. Type II respiratory failure was seen in 24% of the study

participants. This result aligns well with previous research, showing a prevalence of around 25% for type II respiratory failure in COPD patients.[12] The previous results were based on the fact that a number of key factors contribute to type II respiratory failure in COPD including airway obstruction, reduced lung elasticity, and alveolar damage causing elevated levels of carbon dioxide and reduced oxygen in the blood stream. However, the small differences observed between this study and others can be attributed to the variability in contributing factors across different regions and populations. One notable factor is smoking behaviour. Smoking rates can differ significantly between countries, and regions with higher smoking prevalence are likely to experience a greater burden of COPD and its complications, including type II respiratory failure.[13] Environmental factors also play a crucial role in COPD development. Differences in exposure to indoor and outdoor pollutants, occupational hazards, and biomass fuels can influence COPD prevalence and its severity.

Genetic predisposition is another important factor influencing COPD prevalence and severity. Populations with different genetic backgrounds may have varying rates of respiratory failure in COPD patients. For example, Alpha-1 antitrypsin deficiency (A1ATD) is caused by a mutation in the *SERPINA1* gene, leading to deregulation of neutrophil elastase –a protease enzyme. This causes lung tissue degradation and emphysema. Cigarette smoke can hasten lung harm in individuals with A1ATD. Overall, that study emphasizes the importance of understanding the multiple factors contributing to type II respiratory failure in COPD patients.[14]

The higher prevalence of type II respiratory failure in male COPD patients observed in this study is aligned with findings from international studies.[15] This data supports previous findings that COPD is more prevalent among males, primarily due to higher smoking rates, a leading risk factor for the disease in men. While the prevalence in our study is slightly higher than international results, this may be attributed to high smoking rates among males in our country. Other factors such as socioeconomic disparities and lifestyle choices impacting COPD outcomes might also have contributed these findings. It is worth mentioning that male-dominated occupations in Pakistan may expose men to various pollutants, dust, and fumes, increasing the risk of developing COPD and thus type II respiratory failure.[16]

Another study demonstrated a correlation between age of patient and type II respiratory failure in COPD which is consistent with previous research.[17] The natural aging process contributes to hyper-inflated alveoli and reduced elastic recoil, leading to CO_2 retention and respiratory failure. Reduced physical activity and a sedentary lifestyle with age may further exacerbate this condition.[18] Interestingly, the proportion of patients over 55 years was lower than in international studies, potentially influenced by quality of life, life expectancy factors, early smoking practices, and an increased frequency of respiratory infections during childhood and adolescence in our country. These factors collectively contribute to the observed differences in age-related prevalence of type II respiratory failure in COPD patients.[19]

Regarding the COPD severity staging based on the GOLD classification, the study did not find a statistically significant association with the prevalence of type II respiratory failure. These results support previous research findings, showing considerable inter-individual variation and overlap between different stages of COPD for various health outcomes.[20]

The results also indicate lack of association between BMI and Type II respiratory failure in COPD, which is not consistent with a previous study[21] on effects of BMI on type II respiratory failure in COPD. This may be attributed to the small sample size, which limited the power of analysis to detect significant associations. Factors like smoking history, physical activity levels, medication usage, and other health conditions could have acted as confounding variables, influencing the relationship between BMI and COPD outcomes.[22] The participants' diverse characteristics, including varying disease severities, co-morbidities, and lifestyle, might have further masked any potential association between BMI and Type II respiratory failure. To better understand the link between BMI and COPD outcomes, larger and more comprehensive studies controlling for confounders and exploring different patient subgroups are needed, along with meta-analyses to strengthen the overall evidence base.

CONCLUSION

This study highlights the significant frequency of type 2 respiratory failure in COPD patients. Type 2 respiratory failure is more prevalent in elder patients above 55 years of age, and is more common among males. The severity of COPD as determined by GOLD staging does not appear to have a direct relationship with the occurrence of type 2 respiratory failure.

LIMITATIONS OF THE STUDY

This study did not address the underlying causes of COPD. Patients were labelled under the umbrella term COPD and not further classified as chronic bronchitis and emphysema. Addition of these would have made it more comprehensive.

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Address for Correspondence:

Dr Mohsin Ali, PhD Scholar, Department of Pharmaceutical Sciences, Ghent University, Belgium. **Cell:** +32-499-313465

Email: Mohsin.Ali@ugent.be

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JD: Principal Author
HM: Data Collection
A: Data Collection, Study design, Script writing
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ORIGINAL ARTICLE

PROTECTIVE EFFECTS OF SILYMARIN AND CHOLCALCIFEROL ON ISONIAZID INDUCED HEPATOTOXICITY IN MICE

Rabia Iftikhar Malik, Akbar Waheed*, Salma Salim, Fatima Muhammadi*, Nimra Ijaz***, Uzma Naeem***

Department of Pharmacology, Shifa College of Medicine, Shifa College of Medicine, Islamabad, *Islamic International Medical College, Rawalpindi, **Mohi-ud-Din Medical College, Mirpur AJK, ***Fazaia Medical College, Air University, Islamabad, Pakistan

Background: Hepatotoxicity is the gravest concern associated with use of anti-tuberculous drugs. The objective of this study was to evaluate the individual and combined hepatoprotective effect of silymarin and cholecalciferol in isoniazid induced hepatotoxicity. **Methods:** This animal experimental study was conducted at the Department of Pharmacology & Therapeutics, and Multidisciplinary Research Laboratory, Islamic International Medical College, in collaboration with National Institute of Health, Islamabad, Pakistan. Fifty adult Balb-C mice were included in this study. They were distributed into 5 groups. Each group contained 10 mice. Group 1 was normal control, Group 2 disease control, and Group 3, 4, and 5 were experimental groups. Except Group 1, all other groups were given isoniazid (150 mg/Kg) and only Group 2 was not fed with any drugs. Group 3 received silymarin (50 mg/Kg dissolved in physiological saline) through intragastric gavage for 28 days. Group 4 was given Vitamin D (1,000 IU/Kg) for 28 days. Group 5 was given isoniazid (150 mg/Kg) along with silymarin and Vitamin D for 28 days. Serum ALT and bilirubin levels were estimated on day 0, 14, and 28. **Results:** As compared to Group 2, Group 3 to Group 5 showed a lower rise in serum ALT and bilirubin (p<0.001). Group 4 and 5 showed significantly reduced biochemical markers (ALT and bilirubin) (p=0.001). **Conclusion:** Silymarin and cholecalciferol effectively and synergistically ameliorate hepatotoxicity induced by isoniazid. Silymarin offers better hepatoprotection than cholecalciferol in isoniazid induced hepatotoxicity.

Keywords: Silymarin, Hepatotoxicity, Isoniazid, Cholecalciferol

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INTRODUCTION

Tuberculosis (TB) is a leading cause of mortality and morbidity all over the world. Among the countries with the highest burden of TB, Pakistan is ranked 5th.[1] Multi-drug resistant (MDR) tuberculosis is one of the greatest challenges faced, and an incidence of 342 per 100,000 population has been recorded in Pakistan.[2]

Hepatotoxicity is the gravest concern associated with anti-tuberculous drug usage. They remain the leading cause of idiosyncratic hepatotoxicity worldwide. Factors that determine the development of hepatotoxicity depend on the drug regimen used, properties of the cohort under study and the threshold used to define hepatotoxicity along with its reporting and monitoring methodology.[3] Hepatotoxicity secondary to anti-TB drugs has been reported in 5–28% of people treated with anti-TB drugs. Standard treatment of TB is a combination regimen consisting of isoniazid, rifampin, pyrazinamide and ethambutol. The first three drugs are known hepatotoxic agents and drug induced liver injury (DILI) is the most serious adverse effect seen in patients taking anti-TB treatment (ATT).[4] Anti-TB drug induced hepatotoxicity has been defined by the Japanese Society of TB based on levels of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total bilirubin values. DILI is defined as elevation of peak serum aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT) of more than twice the upper limit of normal.[5] Even though the global incidence of tuberculosis is on the decline, the global disease burden is still considerable (~9 million cases and ~1.5 million deaths in 2018), incidence of tuberculosis and drug resistance are rising in some parts of the world like Africa.[3]

Isoniazid and pyrazinamide are well-known hepatotoxic drugs often used in combination. Pyrazinamide (PZA) induces serious liver injury, but the exact mechanism of PZA-induced hepatotoxicity remains controversial. Endoplasmic reticulum stress-caused cell apoptosis plays a critical role in the development of DILI.[6] Isoniazid is metabolized by N-acetyltransferase 2 (NAT-2) to acetylhydrazine and diacetylhydrazine.[7] Diacetylhydrazine is nontoxic and is readily eliminated from the body.[8] Vitamin D, specifically the biologically active vitamin D metabolite 1, 25-dihydroxyvitamin D [Calcitriol: 1, 25 (OH)2D], is known to exert important physiological effects in addition to well-known effects on calcium metabolism. Relation of Vitamin D to the severity of liver disease has been reported among liver disorders other than ATT-Liver Disease.[9] Patients with non-alcoholic steatohepatitis (NASH) have significantly lower levels of Vitamin D. In addition, Vitamin D deficiency is associated with the histopathological severity of

NASH.[10] Animal studies demonstrate that elevated level of Vitamin D using phototherapy results in lower severity of non-alcoholic fatty liver disease as indicated by less necroinflammation, fibrosis, and apoptosis.[11]

Silymarin, a flavonoid from 'milk thistle' (*Silybum marianum*) plant is used almost exclusively for hepatoprotection. Silymarin manifests hepatoprotection by scavenging free radicals, raising the glutathione content, inhibiting lipid peroxidation, and restoring the function of enzymes, thereby generating membrane stabilization and preventing toxic metabolic liver injury.[12] Cholecalciferol is a readily available and essential vitamin involved in a number of physiological processes. It has shown to exert hepatoprotective effects. Experimental models showed that Vitamin D stops the proliferation of main hepatic stellate cells, decreases expression of collagen, and halts thioacetamide-induced fibrosis in liver.[10] According to Hasanain *et al*[13], patients given adjuvant cholecalciferol supplement showed significantly decreased incidence of ATT-Liver Disease as opposed to those without the supplementation (13.3 *vs* 5.3%, p=0.001). The current study compared the effects of vitamin D with a known hepatoprotective agent silymarin. It also aimed to see if a combination of silymarin and vitamin D has synergistic effects.

MATERIAL AND METHODS

This study was performed from Sep 2020 to Aug 2021 in the Department of Pharmacology, Islamic International Medical College after getting approval from the Institutional Review Committee (Approval No. Riphah/IRC/20/247). This study was conducted on a total of 50 male albino Balb/C mice divided into 5 groups each having 10 mice. Sample size was calculated using Resource Equation method.[14] Mice included in study had approximately 30–50 g weight and normal LFTs pre-intervention.[15]

Mice were placed in well-aerated cages for acclimatization, at room temperature of 22±2 °C, and a 12-hour light/dark cycle was maintained. Group 1, the control group, consumed a normal diet and tap water throughout the experiment while group 2, 3, 4, and 5 were given isoniazid in dose of 150 mg/Kg. Group 3 received silymarin also in a dose of 50 mg/Kg dissolved in 0.9% physiological saline solution.[15,16] Group 4 received vitamin D 1,000 IU/Kg for 28 days.[18] Group 5 received both isoniazid and vitamin D in the aforementioned doses along with silymarin for 28 days. On day 0, blood samples were taken for a baseline evaluation, and on day 14, a second sampling was done for the confirmation of hepatotoxicity in groups 2, 3, and 4. The final sampling took place on day 28 of the experiment.[16] BALB/C mice were chosen because the characteristics that these mice possess make them an ideal model for hepatotoxicity studies. Parameters like includes induction of injury to the liver in a huge percentage of these mice, low cost of the animal, as well as easy management.[17]

ALT and serum bilirubin levels were estimated. Serum ALT was estimated by IFCC method. Total bilirubin was estimated according to Calorimetric method. The statistical analysis was done on SPSS-24. Values were expressed as Mean±SD. The statistical significance of the differences of various quantitative changes between the experimental and control groups were evaluated using one-way ANOVA followed by Tukey's Honestly Significant Difference post hoc test for multiple comparisons. The difference was regarded statistically significant at $p \leq 0.05$.

RESULTS

Table-1 and 2 show comparison of mean differences of the groups. The results of group 3, 4, and 5 were compared to group 2. In silymarin plus cholecalciferol treated group 5, there were substantially low levels of ALT and billirubin as compared to group 2, the disease control group. The results also imply that the synergistic effect of silymarin plus cholecaciferol as a treatment tool for hepatotoxicity is greater than the effect of each drug individually in groups 3 and 4 respectively. The significant results are presented and compared with other groups in the Tables below.

Table-1: Post-Hoc Tukey test showing comparison of ALT between groups on day 28

Groups	Mean difference	*p*
1 *vs* 2	119.33	0.000*
1 *vs* 3	27.33	0.000*
1 *vs* 4	33.16	0.001*
1 *vs* 5	19.83	0.965
2 *vs* 3	92.0	0.000*
2 *vs* 4	92.0	0.001*
2 *vs* 5	86.16	0.000*
3 *vs* 4	99.5	0.764
3 *vs* 5	5.8	0.000*
4 *vs* 5	13.33	0.006*

*Significant

Table-2: Post-Hoc Tukey test showing comparison of bilirubin between groups on day 28

Groups	Mean difference	*p*
1 *vs* 2	0.09	0.000*
1 *vs* 3	0.093	0.000*
1 *vs* 4	0.093	0.001*
1 *vs* 5	0.093	0.867
2 *vs* 3	0.093	0.000*
2 *vs* 4	0.093	0.001*
2 *vs* 5	0.093	0.000*
3 *vs* 4	0.093	0.564
3 *vs* 5	0.093	0.000*
4 *vs* 5	0.093	0.006*

*Significant

DISCUSSION

The use of silymarin 100 mg/Kg alone in group 3 and in combination with cholecalciferol in group 5 resulted in prevention of hepatic damage in the mice. This

protective effect was due to its membrane stabilizing function, which keeps intracellular enzymes from leaking out. Because of its antioxidant qualities, it also stimulates phase 2 detoxification pathways. There was a significant difference in the ALT and bilirubin levels between the disease control group 2 and the groups 3 and 5 which received silymarin. These findings are consistent with the study findings of Nasim Ilyas *et al*[16] who demonstrated hepatoprotective effects of silymarin and garlic on isoniazid induced hepatotoxicity.

The decreased levels of ALT and bilirubin of group 3 in our study are supported by Sude Emnizade *et al*[19] who showed that silymarin protects the liver against toxic effects of anti-tuberculosis drugs. They found that silymarin played a hepatoprotective role in hepatotoxicity induced by anti-tuberculous drugs such as isoniazid, rifampicin and pyrazinamide. This effect was demonstrated in a manner similar to our study, with a fall in ALT and bilirubin levels.

Our study showed that silymarin has superior activity in protecting liver against DILI as compared to Vitamin D, as shown by the lower levels of ALT and Bilirubin in group 3 in comparison to group 4. These findings are supported by the findings of Chote Luangchosiri *et al*[21] who conducted a double-blinded randomized controlled trial of silymarin for the prevention of anti-tuberculosis drug-induced liver injury and concluded that silymarin played a protective role demonstrated by lowered ALT levels. Our study shows hepatoprotective effects of silymarin by a fall in ALT and bilirubin levels which was also seen by Reddy MK *et al*[22]. Their study was based on comparing the hepatoprotective effects of silymarin and rutin.

Our findings in group 4 demonstrated that Vitamin D played a hepatoprotective role in INH induced hepatic insult. It also demonstrates that a combination of silymarin and Vitamin D as administered in group 5 is superior in hepatoprotective effects as compared to either silymarin or Vitamin D alone. This finding was supported by a fall in both ALT and bilirubin levels. Therefore these two compounds have a synergistic effect on hepatoprotection (p=0.006) to support these findings we can look at the study conducted by Hasnain AF *et al*[13]. It showed that cholecalciferol played a hepatoprotective role in patients given standard anti-tuberculosis therapy. They found that patients who were given concurrent Vitamin D with ATT therapy had significantly lower levels of aminotransferases as opposed to patients who received ATT alone, especially for ALT.

Wang YQ *et al*[20] recently conducted a study on the deleterious effects of Vitamin D deficiency on acetaminophen exposed mice. Their study concluded that APAP-induced elevations in ALT and AST were exacerbated in mice fed Vitamin D deficient diet. APAP-induced liver necrosis was exacerbated in mice that were fed Vitamin D deficient diet as well. These findings were proved using parameters similar to ours, namely serum ALT and bilirubin examination.

CONCLUSION

Silymarin and cholecalciferol effectively and synergistically ameliorate hepatotoxicity induced by isoniazid. Silymarin offers better hepatoprotection than cholecalciferol in isoniazid induced hepatotoxicity.

RECOMMENDATIONS

Our study is small scale study which involved only 50 mice. Study does not include total protein level, coagulation profile and prothrombin activity. AST level and oxidative stress is not measured in this study. These parameters could not be delved into because of cost and time limitation.

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Address for Correspondence:

Dr Rabia Iftikhar Malik, Senior Lecturer, Department of Pharmacology, Shifa College of Medicine, H-8/4 Islamabad, Pakistan. **Cell:** +92-336-5210716
Email: rabiaift@outlook.com

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Contribution of Authors:

RIM: Manuscript writing
AW: Data analysis
SS: Study design
FM: Data acquisition
NI: Methodology
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ORIGINAL ARTICLE

EFFECT OF ITEM WRITING FLAWS IN MULTIPLE CHOICE QUESTIONS ON STUDENTS' ACHIEVEMENT GROUPS AND RELIABILITIES OF TESTS IN AJK MEDICAL COLLEGE

Sarmud Latif Awan, Shagufta Manzoor*, Irum Gillani, Ziyad Afzal Kiyani, Sahar Khurshid, Humza Farooq*****

Department of Surgery, Abbas Institute of Medical Sciences/AJK Medical College, Muzaffarabad, *Department of Anatomy, AJK Medical College, Muzaffarabad, **Department of Public Health and Community Medicine, Health Services Academy, Islamabad, ***Department of Surgery Shifa International Hospital, Islamabad, Pakistan

Background: Multiple-choice question (MCQ) is a commonly used tool for written assessment. Purpose of this study was to determine the effect of item writing flaws in MCQs on students' academic achievements and test reliabilities. **Methods:** This study was conducted at AJK Medical College Muzaffarabad from Dec 2017 to Jun 2019. Ten summative tests were included. The item review committee examined every MCQ for errors in item writing. The initial tests with all items were deemed to be flawed tests. The outcomes of these tests were assessed and students were ranked into three achievement groups based on their scores, i.e., high, moderate, and low achievers with scores of >79.9, 50–79.9, and <50% respectively. Once the review board eliminated the flawed items, the scores of each test (the standard test) were calculated, compared to the flawed tests and its impacts were evaluated between three achievement groups. The post-exam analysis was done using the optical mark reading classic-4 programme. Data were analyzed using SPSS-25. **Results:** In only test No. 6 the null hypothesis, i.e., there is no effect of flawed items on students' academic achievements was statistically rejected (p<0.05). Among high, moderate and low achievers between flawed and standard tests, moderate achievers and low achievers had statistically significant correlation (p<0.003 and <0.044 respectively). The flawed tests had better reliabilities than standard tests with statistically significant difference (p<0.012**) Conclusion:** Flawed items negatively affect high and moderate achievers and affect low achievers positively. Flawed tests had better reliabilities.

Keywords: Academic achievements, flawed items, MCQs

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INTRODUCTION

Students' learning is greatly influenced by assessment which also helps to accomplish curricular goals. Multiple-choice question (MCQ) is a commonly used tool for written assessment in health professions education.[1] Properly constructed MCQs can test different levels of cognitive knowledge from recall, comprehension to application, synthesis and analysis.[2] MCQs tests also discriminate between high and low achieving students.[3] However, construction of a high quality MCQ is a time consuming, laborious and taxing task, even for a properly trained medical educationist.[4]

Few institutions in Pakistan have properly trained medical educationists with formal training in writing MCQs. A large number of in house MCQs are developed by faculty with little or no training; hence, these are mostly of low quality. There are several guidelines for construction of high quality MCQs.[5] A detailed taxonomy of 31 item-writing rules has been described.[6] The evidence-based recommendations for the construction of one best MCQ are often violated by item writers which leads to the production of flawed MCQs items with adverse effects on student's academic achievements.[6]

Medical education is being supervised by PMDC in Pakistan. But there is no organized mechanism for supervision and evaluation of standards of examinations by PMDC or any other supervisory authority in Pakistan. In view of scarcity of medical educationists and established medical education departments, local faculty members in different medical institutions are at liberty to develop MCQs in their own way. The quality of MCQs is primarily dependent on the experience and training of faculty, which varies from institution to institution.

This study will help in determining the effect of item writing flaws in multiple choice questions in basic and clinical sciences on students' academic achievements and on test reliabilities rectifying the need for creation of some mechanism by regulatory authorities to supervise the quality aspects of MCQs based examinations in medical institutions of Pakistan.

METHODOLOGY

This study was a non-experimental descriptive study carried out from Dec 2017 to Jun 2019 in AJK Medical College, Muzaffarabad. This study was approved by the ethical committee of AJK Medical College. Ten summative and end-of-block assessments

in AJK Medical College were included in this study. These tests were taken from assessments of 1st, 2nd, 3rd, 4th and 5th year classes with two tests from each class. Modules included in study were those in which college faculty had maximum input in terms of MCQ construction and these were the part of internal assessment that constituted 30% of final professional summative assessment.

All MCQs were reviewed by the item review committee for item writing flaws in the examination department of AJK Medical College. Tests from summative end of block assessment with post-examination analysis statistical data interms of reliability of test, difficulty index, point biserial and discrimination indices of items, with 90 or more student per test, 50 or more number of MCQs written by local faculty per test, with reliability of 0.6 or greater were included in the study. The initial result of each test (flawed test with all items in the test) was obtained and students grouped accordingly into high, moderate and low achieving groups. Each test item was reviewed by review committee. The review committee comprised of one writing expert and one relevant subject specialist.

There was only one item writing expert who was the permanent member of each review committee. There were different subject specialists for each MCQ paper from different disciplines. Flawed items were then removed from the tests by review committee.

The scores of each test (standard test without flaw items) were then determined and students were graded into high, moderate and low achievers. The scores of each flawed test and standard test were compared and their effects were determined in three achieving groups. Optical mark reading (OMR) classic-4 software was used for post-exam analysis of flawed items.

RESULTS

The observed differences in the number of students in different achievement groups in flawed and standard tests in this study are shown in Table-1. In one test (tests No. 6) the null hypothesis, i.e., there was no statistical significant association of flawed items to achievement groups was rejected with 95% confidence interval. In all other tests (Test 1, 2, 3, 5, 7, 8, 9, 10) null hypothesis could not be rejected on statistical basis. These results showed that there was significant association of presence or absence of flawed items to achievement groups in one test. There was no statistical significant association of flawed items to achievement groups in nine tests in this study. (Table-1).

Cumulative differences were observed in each achievement group of students in all tests. These observed differences are summarized in Table-2.

Table-1: Differences in the frequency of achievement groups in flawed and standard test

Achievement Groups	Pass	Fail	High	Moderate	Low	*p*
Test-1						
Flaw	86	20	0	86	20	0.116
Standard	84	22	4	80	22	
Test-2						
Flaw	89	9	2	87	9	0.09
Standard	90	8	9	81	8	
Test-3						
Flaw	60	28	2	58	28	0.474
Standard	67	21	3	64	21	
Test-4						
Flaw	84	11	4	80	11	0.432
Standard	89	6	5	84	6	
Test-5						
Flaw	82	11	2	80	11	0.46
Standard	78	15	4	75	15	
Test-6						
Flaw	77	18	7	70	18	0.029
Standard	89	6	10	79	6	
Test-7						
Flaw	81	14	5	76	14	0.406
Standard	86	9	8	78	9	
Test-8						
Flaw	82	24	2	80	24	0.219
Standard	90	16	5	85	16	
Test-9						
Flaw	73	14	6	67	14	0.667
Standard	77	10	7	70	10	
Test-10						
Flaw	73	14	9	64	14	0.346
Standard	71	16	4	67	16	

Table-2: Number of students in achievement groups in flawed and standard test

Achievement group	Flawed tests	Standard tests
High achievement group	39	59
Moderate achievement group	748	763
Low Achievement Group	163	129

Cumulative differences of achievement groups (high, moderate, and low)

The high achievers in flawed tests were 39, and in standard tests they were 59. There was a difference of 20 students. Inclusion of flawed items in these tests negatively affected scores of high achievers. There were increase number of high achievers in standard tests than flawed tests but the correlation was not statistically significant (p>0.05). The moderate achievers in flawed tests were 748 and in standard tests there were 763. There was a difference of 15 students. Inclusion of flawed items in these tests negatively affected scores of moderate achievers.

There were increase number of moderate achievers in standard tests than flawed tests and the correlation was statistically significant (p=0.003). The low achievers in flawed tests were 163 and in standard tests there were 129. There was a difference of 34 students. Inclusion of flawed items in these tests negatively affected scores of low achievers. There was decrease in number of low achievers in standard tests than flawed tests and the correlation was statistically significant (p=0.044) (Table-3).

Table 3: Correlation of achievement groups between flawed and standard tests

		High achievement students in standard test	High achievement students in flawed test
High achievement students in standard test	Pearson Correlation	1	0.325
	Sig. (2-tailed)		0.360
	N	10	10
High achievement students in flawed test	Pearson Correlation	0.325	1
	Sig. (2-tailed)	0.360	
	N	10	10
		Moderate achievement students in standard test	Moderate achievement students in flawed test
Moderate achievement students in standard test	Pearson Correlation	1	0.829**
	Sig. (2-tailed)		0.003
	N	10	10
Moderate achievement students in flawed test	Pearson Correlation	0.829*	1
	Sig. (2-tailed)	0.003	
	N	10	10
		Low achievement students in standard test	Low achievement students in flawed test
Low achievement students in standard test	Pearson Correlation	1	0.645*
	Sig. (2-tailed)		0.044
	N	10	10
Low achievement students in flawed test	Pearson Correlation	0.645**.	1
	Sig. (2-tailed)	0.044	
	N	10	10

*Correlation is significant at the 0.01 level (2-tailed), **Correlation is significant at the 0.05 level (2-tailed).

The null hypothesis could not be rejected for high achievement group in this study. There were observable difference in high achievement group in flawed and standard test but this difference was not statistically significant. There was statistically significant association of flawed items in moderate and low achievements groups. The null hypothesis was rejected in these groups with p=0.003 and 0.044 respectively. Kuder-Richchardson-20 Formula (KR-20) was used to determine the reliabilities of flawed and standard tests. The reliabilities ranged from 0.6 to 0.78. The mean of reliabilities for flawed tests and standard tests were 0.72 and 0.65 respectively. In all tests reliabilities of flawed tests were better than standard tests. (Table-4).

Table-4: Reliabilities of flawed and standard tests

Reliability of Tests	Kuder-Richardson Formula 20
Test-1	
Flawed	0.66
Standard	0.60
Test-2	
Flawed	0.74
Standard	0.72
Test-3	
Flawed	0.68
Standard	0.62
Test-4	
Flawed	0.78
Standard	0.72
Test-5	
Flawed	0.69
Standard	0.63
Test-6	
Flawed	0.78
Standard	0.66
Test-7	
Flawed	0.78
Standard	0.66
Test-8	
Flawed	0.68
Standard	0.62
Test-9	
Flawed	0.66
Standard	0.60
Test-10	
Flawed	0.76
Standard	0.71

Mann Whitney U test was used to determine statistical significance between reliabilities of flawed and standard test. The difference in reliabilities of flawed and standard tests was found to be statistically significant (p=0.012) in the current study, so null hypothesis, i.e., there is no significant difference in reliabilities of flawed and standard test, could be rejected. (Table-5).

Table-5: Man-Whitney test statistics

Test parameters	Reliabilities
Mann-Whitney U	17.000
Wilcoxon W	72.000
Z	-2.512
Asymp. Sig. (2-tailed)	0.012
Exact Sig. [2*(1-tailed Sig.)]	0.011[a]

[a]Not corrected for ties

DISCUSSION

In this study, the students were grouped into high, moderate, and low achievement group on the basis of their performance. The presence of flawed items in the tests had a negative effect on the results. Exclusion of flawed items led to increase in number of students from in high and moderate achievement groups, and decrease in number of students in low achievement group. These findings are similar to studies by Downing[7,8] and Tarrant[9]. Inclusion of flawed items in the test not only contorted the pass/fail decisions but also negatively affected the process of awarding grades to the students in the test. An observable number of students could not achieve >80% score because of the flaw items in the test. Similarly, 34 students who deserved to be in moderate achievement group fell in low achievement group.

The prime objective of the assessment is not to award grades to the students but to differentiate between high and low achieving students. In our study, the actual boundaries of the three achievement groups of students were distorted by these flawed items, as high performing students gave the impression of being moderately

performing students and moderately performing students as low performing students. Inclusion of flawed items in the tests greatly compromised the authenticity of grading decision in the assessment.

According to Axelson *et al*[10], the reliability of a test is an estimate of proportionate amount of random error in the data. Reliabilities of all tests (standard) in this study got decreased when flawed items were removed from the test. There was a decrease in the reliability of 10 standard tests after removal of flawed items from the tests. These differences were statistically significant in this study. In a study by Downing[7], there was no difference in the reliability of flawed and standard scales. Tarrant *et al*[9] used KR-20 for measurement of internal consistency of 10 tests. The KR-20 ranged from 0.54 to 0.87. The reliability estimates in our study were similar to findings of KR-20 reliability. The reliability of 8 (out of 10) standard tests in Tarrant[9] study was lower than the reliability of total (flawed) scale even after correction for the length of tests. We also had similar findings where reliability of 10 standard tests was lower than the reliability of flawed tests. Two important determinants of the reliability of a written test are the length of test and performance of items on test.[10] When these flawed items were removed from the test, the length of the test got reduced and as a result the reliabilities of these tests (standard) also decreased. There was an observable reduction of reliability where maximum and minimum items were removed from the test. The reliability of tests was more influenced by the length and number of items in a test. The reliability of standard tests reduced after removal of flawed items with acceptable psychometrics.

CONCLUSION

The use of flawed items in the assessment results in negatively affecting high and moderate achievers and positively affecting low achievers. Inclusion of flawed items in tests greatly compromised the authenticity of grading decision in the assessment. Overall reliabilities of flawed test were greater than standard tests.

RECOMMENDATIONS

This was a small study, a way forward but certainly not enough to resolve all controversies. A larger, preferably multi-centre, randomized control study will be required to resolve the issue. Faculty development programmes can provide the platform for raising the quality of assessment in medical institutions. It is the responsibility of institution especially Medical Education Department to identify and rectify these commonly repeated flaws during faculty training.

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Address for Correspondence:

Dr. Sarmad Latif Awan, Associate Professor, Department of Surgery, Abbas Institute of Medical Sciences/AJK Medical College, Muzaffarabad. **Cell:** +92-334-5285882
Email: sarmadawan@hotmail.com

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ORIGINAL ARTICLE

MENTAL HEALTH AND RESILIENCE AMONG PAKISTANI WOMEN SUFFERING FROM POLYCYSTIC OVARY SYNDROME

Wardah Ishfaq, Rabia Mushtaq*

Department of Psychology, International Islamic University, and Shifa Tameer-e-Millat University, Islamabad,
***Department of Psychology, International Islamic University Islamabad, Pakistan**

Background: Polycystic Ovary Syndrome (PCOS) is associated with multiple mental health issues, however resilience can work as a buffer against mental illnesses. The objective of the present study was to investigate the relationship between mental health and resilience and to compare mental health and resilience between working and non-working women suffering from PCOS. **Method:** This cross-sectional comparative study was conducted in the gynaecological wards of government and private hospitals and clinics from January to June 2023. Two hundred women suffering from PCOS were selected through non-probability sampling. The data was collected through two reliable measures: the Mental Health Inventory 38-Urdu (MHI-38) and the Connor Davidson Resilience Scale-Urdu. Data was analyzed on SPSS-26 by means of Cronbach alpha reliability, Pearson correlation, Mean±SD, and *t*-tests. **Results:** Psychometric properties showed high reliability of the study scale for MHI 38-U (α=0.87) and for CDRS-U (α=0.82, $p<0.001$). There was a significantly positive correlation between mental health and resilience ($r=0.85$, $p<0.001$). Non-working women experience lower mental health (Mean±SD 85.37±9.91) than working women (Mean±SD 136.50±12.94), ($p<0.001$). Working women experience higher resilience (Mean±SD 65.06±8.71) than non-working women with PCOS (Mean±SD 47.55±12.17), ($p<0.001$). **Conclusion:** There was a significant positive relationship between mental health and resilience. Non-working women reported compromised mental health and low resilience compared to working women suffering from PCOS.

Keywords: Mental health, resilience, working, non-working, PCOS

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INTRODUCTION

Polycystic ovarian syndrome (PCOS) is a prevailing endocrine disorder in women. In Pakistan the prevalence of PCOS is much higher (52%) as compared to other South Asian and Western countries (26%).[1] PCOS has significant and varied clinical effects, including those on the reproductive system (infertility, hyperandrogenism, hirsutism), the metabolic system (insulin resistance, impaired glucose tolerance, type 2 diabetes mellitus, adverse cardiovascular risk profiles), and the psychological system (increased anxiety, depression, and worsened quality of life).[2] Adults with PCOS are more likely to experience hirsutism, menstrual irregularity, and infertility[3], whereas adolescents and young women with PCOS are more likely to experience weight issues[4,5]. PCOS is associated with obesity, which may raise the incidence of eating problems in women. The presence of obesity, particularly central obesity, is a prevalent characteristic of PCOS that exacerbates the phenotypic.[6] There is a high prevalence of depression among individuals diagnosed with PCOS.[7] Most obese persons have mood disorders such as depression.[8]

In a study conducted by Adali *et al*, it was observed that individuals diagnosed with PCOS had considerably higher values of body mass index (BMI) and waist-to-hip ratio (WHR) compared to the control group. Their results indicated that patients with PCOS experienced significantly elevated levels of emotional distress and depression.[9] Resilience is an essential resource for the soul energy to prevent and alleviate mental health problems such as depression, anxiety, and stress. Resilience is the capacity to recover and thrive.[10] Individuals exhibiting high levels of resilience have low levels of emotional and behavioural distress such as sadness, anxiety, and stress. Resilience is linked to reduced psychological problems and helps the individual to cope better with adverse situations.

It has been indicated that working women are frequently exposed to a range of distressing human experiences and hardships, which have the potential to adversely impact the psychological well-being of these individuals. To effectively manage the various internal and external challenges they face, individuals must actively mobilize and utilize their psychological resources such as resilience.[11] Foumani *et al*, examined how personal traits affect resilience and happiness in working and non-working women residing in Tehran. The findings of their study indicate that personality factors play a significant role in influencing specific aspects of happiness and resilience among females. Women with balanced personalities are therefore more likely to be content and resilient.[12]

People who have strong professional networks also tend to have supportive connections with one another. In an ideal scenario, it is expected that all collegial relationships would possess a certain level of

nurturing. The provision of nurturing and having cooperative connections are essential for individual's well-being. A supportive atmosphere can facilitate the establishment of mutually beneficial and nurturing connections among workers.[13] McGee believes that guidance and support from colleagues and the growth of resilience can reduce psychological distress in working women.[14] Previous research studied resilience and mental health issues like psychological distress among working and non-working women in the absence of medical problems.

The objectives of this study were to examine the correlation between mental health and resilience in women diagnosed with PCOS, and to evaluate levels of mental health and resilience between employed and unemployed women affected by PCOS.

METHODOLOGY

A sample of 200 (108 working, 92 non-working) women suffering from PCOS was taken through purposive sampling from different government and private hospitals and clinics in Rawalpindi and Islamabad. The age range of the sample was 18–45 years. The study was approved by the Ethical Review Board of International Islamic University, Islamabad. Permission was taken from respective clinics and hospitals, and the participants were briefed about the purpose and objectives of the study. All the participants extended written informed consent. A demographic sheet and two scales, Mental Health Inventory-38-Urdu (MHI-38)[15], having two sub-scales, the psychological distress scale and psychological well-being scale, and Conner Davidson Resilience Scale-Urdu[16] were given to participants. Computing scores of Mental Health Inventory were used for analysis. The score of the sub-scales can be used independently. Higher scores indicate better mental health and resilience.

Data were analysed on SPSS-26. Mean and Standard Deviations were calculated. Pearson correlation and Student's *t*-test were applied.

RESULTS

Out of 200 women suffering from PCOS 106 (55%) were working and 94 (45%) were non-working. Pearson correlation showed that mental health and resilience were significantly and positively correlated with each other (r=0.82, p<0.001) (Table-1).

Mean, Standard Deviation and *t*-values for women with PCOS with respect to occupational status are shown in Table-2. Non-working women had lower mental health (Mean±SD 85.37±9.91) than working women (Mean±SD 136.50±12.94), (p<0.001). Working women experience higher resilience (Mean±SD 65.06±8.71) than non-working women with PCOS (Mean±SD 47.55±12.17), (p<0.001).

Table-1: Correlation matrix between mental health and resilience among women with PCOS (n=200)

Variables	α	1	2
Mental Health Inventory-38	0.87	-	0.85*
Resilience	0.82	-	-

*p<0.001

Table-2: Comparison of mental health and resilience among working and non-working women with PCOS

Variables	Working (n=108)	Non-working (n=92)	p	Cohen's d
Mental health	136.50±12.94	85.37±9.91	0.000	0.98
Resilience	65.06±8.71	47.55±12.17	0.000	1.48

DISCUSSION

The results affirm that mental health and resilience have a positive correlation which means that mental health level affects positively the resilience capacity among women suffering from PCOS. Previous studies also corroborate this result as students with high levels of resilience experience less psychological distress than those with low levels of resilience.[17] Recent studies in the time of COVID-19 also show a similar positive correlation between mental health and resilience.[18] Verdolini *et al* also discovered a noteworthy negative correlation between resilience and psychological problems during the COVID-19.[19] Moreover, survey studies have demonstrated a negative correlation between resilience and psychological distress among physicians[20] as well as depressive symptoms in the general population[21]. Individual interaction, personality traits, experience, family, age, educational level, and human resources, all contribute to resilience.[22] Findings also indicate that working women have higher mental health and high resilience. Women who are employed are typically content, mature to handle interpersonal relationships, passionate, temperately practical, consistent, sheltered, naive, forgiving, joyous, and optimistic. In general, non-working women have a lower sense of well-being than working women.[23]

The prevalence of PCOS continues to rise, and the associated risk factors are severe and long-lasting. Women with PCOS may have lower self-esteem, a more negative self-image, and higher levels of depression and psychological distress due to hyperandrogenism, physical appearance characteristics such as obesity, hirsutism, cystic acne, seborrhoea, and hair loss, which may affect feminine identity.[24] In Pakistani society, due to the stigma of anovulation and infertility, most women do not seek a diagnosis, and the condition worsens over time.[25] There should be a national awareness program about PCOS, its symptoms, consequences, and management. Women with PCOS should maintain regular contact with their healthcare provider to maintain control over their condition or, if necessary, to change treatment options.

Purposive sampling was used to generate the sample of respondents, which limits the present study's

ability to generalize its findings to the whole population. The data was collected in just two cities (Islamabad and Rawalpindi), thereby limiting its external validity. It is suggested that the study be extended to other areas to increase its reliability and generalizability.

CONCLUSION

The result shows a significant positive correlation between mental health and resilience. Findings also elucidate significant differences on mental health and resilience between working and non-working women suffering from PCOS. On the community and national level, prevention awareness programs and seminars should be organized.

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Address for Correspondence:

Warda Ishfaq, Lecturer, Department of Clinical Psychology, Shifa Tameer-e-Millat University, Islamabad, Pakistan.
Cell: +92-313-5553685
Email: wardah.dcp@stmu.edu.pk

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ORIGINAL ARTICLE

ROLE OF SELF-TALK IN PREDICTING DEATH ANXIETY, OBSESSIVE COMPULSIVE DISORDER, AND COPING STRATEGIES IN THE FACE OF COVID-19 AMONG UNIVERSITY STUDENTS

Iqra Safdar, Ifat Gulab*, Nighat Gul**

MS Student, Riphah International University, Islamabad, *Government Postgraduate College for Women, **Government Girls Degree College No. 2, University of Haripur, Pakistan

Background: The current study examines, the role of self-talk in death anxiety, obsessive compulsive disorder (OCD), and coping strategies faced during COVID-19 by university students. The study was conducted among university students after COVID. **Method:** This study included 300 university students, living within the zones of Haripur, Pakistan. They participants were chosen using purposive sampling technique. The self-talk scale (STS), coping strategies inventory short form (CSISF), death anxiety scale (DAS), and Yale-Brown Obsessive-Compulsive Scale (YBOCS) were utilized for assessment. Information was analysed with Student's *t*-test and simple linear regression. **Results:** The female students significantly scored higher on STS, DAS, and CSISF, while male students scored higher on YBOCS. The regression test indicated that self-talk was a significant predictor of coping strategies and death anxiety among university students. **Conclusion:** Self talk was the predictor of death anxiety, obsessive compulsive disorder and copying strategies during COVID-19 outbreak among students gender wise

Keywords: self-talk, death anxiety, copying strategies, YBOCS, OCD, purposive

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INTRODUCTION

In December 2019, a novel virus infection was, to begin with, detected in the city of Wuhan, China. In 1.5 months the infection named corona virus started to dominate worldwide headlines. The World Health Organization (WHO) declared it as a pandemic because of its distant and wide transmission. In Pakistan, the first case of COVID-19 infection was detailed from Karachi on 26 February 2020. Around 9,474 individuals in Pakistan lost their lives due to COVID-19. Since the illness was spreading quickly, it created an environment of fear among individuals of all age groups.[1]

Death anxiety (DA) is defined as, 'a reaction that folks experience when faced with chronic illness, death'. This reaction from the common people shouldn't be astonishing. Fear of dying is proposed to be a central portion of the involvement of being human.[2] Death anxiety is frequently experienced deliberately or unwittingly; it can persuade people to enhance their death anxiety through diversion endeavours to fortify self-esteem.[3] Existential than at phobia is caught on to be the unsurpassed capable frame. Existentialism would propose that citizenry must confront their claim mortality within the occasion that they ought to degree completion. One existential logician, Heidegger, suggested the existential requirement in his book 'Being and Time'. Death anxiety could be an element of ordinary presence when it is a driving force for individuals to a degree completely.[4]

The obsessive-compulsive disorder (OCD) is a psychiatric disorder that involves intrusive and protracted thoughts and time-consuming compulsive behaviours. Obsessions are thoughts that recur and persist despite efforts to ignore or confront them. People with OCD frequently perform tasks, or compulsions, to hunt relief from obsession-related anxiety.[5] Some individuals with OCD perform the compulsive ritual when they feel that it would reduce distress, that relieve the anxiety rooted in particular over-the-top contemplations.[6] Ponders appeared that due to the fear of getting tainted with the corona virus, the person with OCD endeavours to anticipate these inspections and actions but frequently cannot thrive, which eventually causes social and practical disconcerting influences in numerous circumstances. The sprain condition, strong infection, and lethality, in severe cases, can cause mental health problems. Therefore, people use coping strategies to protect themselves and overcome stressful situations. Additionally, living with a relative (within the same house) could also be either a protective factor or a vulnerability factor for developing symptoms, worsening the overall fear of getting contaminated and the importance given to non-public hygiene may have a negative crash on this clinical population.[7]

Coping strategies refer to 'the specific efforts (behavioural and psychological) that people employ to master, tolerate, or minimize stressful events'.[8] They are contemplations and operations that people utilize to influence pressure and push. The COVID-19 flare-up brought about pressure and uneasiness in individuals. The component that appears to be viable inside and out of the three factors of death anxiety, OCD, and

managing stress is that cognition, a person's address to oneself impacts considerations, sentiments, and actions.

Self-talk refers to automatic statements which involve reflective and purposeful ways that people apply to control irrational thinking and establish a healthy psychological state in stressful conditions.[9] Self-talk is also known as internal dialogue. Self-talk can be verbal (in the form of a word) or nonverbal (a thought, a smile, a frown).[10] There is a significant positive relationship between coping strategies and self-talk while, a negative correlation was found among self-talk and death anxiety, and OCD. The regression analysis findings of Damirchi *et al*[6] study indicated that self-talk could predict death anxiety and coping strategies for OCD. Seçer and Ulaş[11] indicated that the effect of corona virus fear on OCD is intervened by emotional reactivity and depression. Another cross-sectional study was conducted among residents in Australia, including patients. Women who had medium to high levels of psychological distress were associated with a higher level of fear and anxiety.[12] Zia and Aslam[13] concluded that death anxiety was positively associated with intrusive and deliberate rumination. Female students scored significantly high on death anxiety, whereas male students significantly scored high on deliberate rumination. Male students scored significantly high on substance abuse coping and avoidance coping strategies whereas, female students scored significantly high on religious coping strategies.[14] Hoelterhoff and Chung's study did not support the significance of religious coping as an important factor; while self-efficacy appeared as significantly related to death anxiety and psychiatric comorbidities.[15]

This study was conducted to investigate the role of self-talk in the prediction of death anxiety and OCD among university students in COVID-19, explore the role of self-talk in coping strategies among university students during the pandemic, and to evaluate the level of death anxiety across gender among university students during COVID-19.

METHODOLOGY

This cross-sectional study was conducted among university students. This study was purely based on a quantitative method. A sample of 300 students (male:female=1:1) of BS and MS were taken from the University of Haripur and Govt. Postgraduate College for Boys, Haripur using a purposive sampling technique. The participants were aged 19–26 years.

The tools used were Death Anxiety Scale[16] which had 15 items with 3 response categories, and has confirmed remarkable internal consistency and stability of the scale as 0.83; Coping Strategies Inventory[15] comprising of 16 items with confirmed remarkable internal consistency and stability as 0.72; the Self-talk Scale of Brinthaupt[17] having 16-item rated on a 6-point Likert scale with internal consistency as 0.79; Yale-Brown Obsessive-Compulsive Scale[18] with 10-items and 5 responses from 0 (no symptoms) to 4 (extreme symptoms), and confirmed remarkable internal consistency and stability of the scale as 0.96.

Data was entered and analysed using SPSS-20. Data were presented in tabular form. Student's *t*-tests and linear regression were used to assess the relationship among variables concerning gender, and $p \leq 0.05$ was considered significant.

RESULTS

The female students significantly scored higher on coping strategies inventory short form (Mean=55.4, $p<0.05$), self-talk scale (Mean=69.2, $p=0.01$) death anxiety scale (Mean=17.8, $p<0.001$) compared to male students. The male students significantly scored higher on YBOCS (Mean=18.6, $p<0.05$) compared to female students (Mean=10.4, $p<0.05$). (Table-1)

As shown in Table-2, R^2 value 0.19 indicates that the predictor variable explained 19% variance in the outcome or dependent variable with F (1, 299)=151, $p<0.001$. Findings indicate that self-talk is a significant negative predictor of death anxiety among university students (β=-0.44, $p<0.001$).

In Table-3, R^2 value 0.33 indicates that the predictor variable explained 33% variance in outcome or dependent variable with F (1, 299)=151, $p<0.001$. Findings indicate that self-talk is a significant positive predictor of coping strategies among university students (β=0.58, $p<0.001$).

Table-1: STS, YBOCS, CSISF and DAS scores in the subjects (n=300)

Logistic Parameters	Female (n=150) Mean±SD	Male (n=150) Mean±SD	*t* (298)	*p*	Cohen's d
CSISF	55.4±15.9	50.8±12.4	2.14	0.03	0.32
STS	69.2±16.6	62.7±14.9	2.46	0.01	0.41
YBOCS	10.4±7.80	18.9±8.18	-0.48	0.02	0.05
DAS	17.8±5.29	14.8±5.15	5.03	0.00	0.57

Table-2: Regression coefficient of self-talk on death anxiety (n=300)

Variables	*B*	β	*SE*
Constant	26.18*		1.18
Self-talk	0.15*	-0.44	0.01
R^2	0.19		

β=Standardized regression coefficient, B=Un-standardized regression coefficient, R^2=proportion of variance, SE=standard error. *$p<0.001$

Table-3: Regression coefficient of self-talk on coping strategies (n=300)

Variables	*B*	β	*SE*
Constant	18.6*		2.84
Self-talk	0.52*	0.58	0.04
R^2	0.33		

β=Standardized regression coefficient, B=Un-standardized regression coefficient, R^2=proportion of variance, SE=standard error. *$p<0.001$

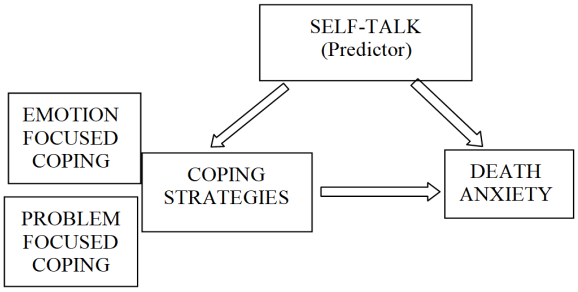

Figure-2: Flow diagram of the variable with reference to Tables-2 and 3

DISCUSSION

Our first hypothesis proposed that levels of death anxiety, self-talk, coping strategies, and obsessive-compulsive disorder will be significantly different in male and female university students. However, female students significantly scored higher on death anxiety, self-talk, and coping strategies than male students, while males scored significantly higher on OCD as compared to female students. Al-Bahram *et al*[19] indicated that female adolescents seem to use maladaptive coping styles more than males. One study results consistent with present research findings indicated that female students scored significantly high on death anxiety, while male students scored significantly higher on substance abuse coping and avoidance coping strategies. Female patients present more OCD symptoms and greater comorbidity with eating and impulse-control disorders as compared to males.[12] Interestingly, Zana's study showed the same result that women have a higher level of fear of death and anxiety.[20]

The second hypothesis proposed that self-talk will significantly predict death anxiety in facing COVID-19 among university students. The result indicated that self-talk is a significant negative predictor of death anxiety among university students. This result was congruent with the study of Damirchi *et al* had reported that the findings of the regression test indicated that, self-talk predicted death anxiety.[6]

The third hypothesis proposed that self-talk will be significantly predicated coping strategy used by university students in facing COVID-19. Our findings indicated that self-talk is a significant positive predictor of coping strategies among students. Damirchi conducted a study and found that self-talk is a predictor of coping strategies (problem-centred style and emotional-coping style).[6]

CONCLUSION

Self-talk serves as a predictor for obsessive-compulsive disorder, death anxiety, and coping strategies among university students during the COVID-19 pandemic. The female students exhibit elevated levels of death anxiety, while male students experience more obsessive-compulsive thoughts.

LIMITATIONS

The following are some shortcomings of this study:

1. The study was limited only to age 19–26 years.
2. As the research was conducted in an educational setting so the findings cannot be used for other settings.
3. This study was performed only among university students of Haripur; it may not be generalized to other areas.

RECOMMENDATION

The university students can get benefits from use of psychological interventions that focus on self-talk since they can help with OCD symptoms, death anxiety, and improving coping mechanisms. Adapting these approaches to address issues unique to a person's gender can increase the efficacy of mental health assistance. To counteract potential negative impacts, educational programs or counselling services that promote constructive self-talk and useful coping methods should be put into place. Studies with a longitudinal design can shed light on how these variables evolve over time, particularly in reaction to current events like the epidemic. By teaching pupils the coping mechanisms, death anxiety and self-talk may decrease.

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Address for Correspondence:

Ms. Nighat Gul, Department of Psychology, Government Girls Degree College No. 2, University of Haripur, Pakistan.
Cell: +92-332-50722147
Email: nighatayub12345@gmail.com

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REVIEW ARTICLE

ASSOCIATION OF SERUM URIC ACID WITH DIABETES MELLITUS TYPE-2: A NARRATIVE REVIEW

Fatima Zehra

Department of Medical Education, Bahria University Health Sciences Campus, Karachi, Pakistan

Diabetes mellitus type 2 (T2DM) is a disease which imposes a great burden on the health resources of the emerging and developing world. It is a global pandemic which leads to chronic complications. Serum uric acid (SUA) is a biomarker that could be of value in detection of early complications and better prognosis in diabetic patients. SUA serves both as a causal factor and disease progression marker in DM, having a causal relationship with diabetic complications. This narrative review aims to determine the association between SUA and T2DM. Research articles from the year 2005 to 2023 were selected for this review. Literature search was conducted from PubMed and Google Scholar which resulted in short listing of 15 articles. It also reflects upon the diabetic complications associated with Hyperuricemia (HUA) and focuses on the health implications of HUA in T2DM patients. SUA and T2DM were negatively associated in 8 research studies and positively associated in 6 studies in which one revealed a partial association, one was prevalent in the female gender only, and one was negatively associated in uncontrolled DM. Another study was associated with both hypouricemia and HUA. SUA levels were moderately increased in moderately elevated glycaemic control and declined in uncontrolled DM with HbA1c levels above 7%.

Keywords: Uric Acid, Glycated haemoglobin, HbA1c, Diabetes mellitus type 2, Biomarker, Complications

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INTRODUCTION

Diabetes mellitus (DM) is a disease which imposes a great burden on the health resources of the emerging and developing world.[1] It is a global pandemic which leads to chronic complications including neuropathy, retinopathy, nephropathy, dyslipidemia, infections, diabetic foot, cardiovascular complications such as stroke and myocardial infarction. The targets of management of diabetes include optimum glycaemic control, which is correlated with HbA1c and is achieved through hypoglycaemic agents. However, a large number of patients are unable to achieve the required target glycaemic control, resulting in progression to chronic complications.[2]

For the purpose of detection of progression of diabetic complications a clinical biomarker is required which could be prognostic in nature. These complications eventually lead to morbidity and mortality in diabetic patients. Hyperuricemia (HUA) is considered as an evolving and rising metabolic disorder, associated with T2DM and obesity. Hence, there is a significant relationship between insulin resistance and HUA. SUA is a biomarker that could assist in detection of early complications and better prognosis in diabetic patients.[3] It could be considered as a predictive marker for renal and cardiovascular complications and as an indicative marker for acute myocardial infarction and chronic coronary syndrome. It could also be considered a therapeutic target in diabetic patients for prevention of disease progression as long term HUA leads to multiple organ damage.[4] Thus SUA serves as both a causal factor and disease progression marker in DM, having a causal relationship with diabetic complications. There is a bidirectional causal effect between HUA and Insulin.[5] It can be used as both a diagnostic and prognostic marker of complications in diabetic patients.[6] It could be recommended as an additional clinical biomarker in all T2DM patients.[7] This could possibly improve patient management in DM. It could also identify potential diabetic patients who could possibly benefit with uricosuric drugs for treatment of HUA. SUA lowering drugs could help prevent diabetic complications, such as diabetic kidney disease (DKD) which leads to the eventual cause of mortality in diabetics.

This narrative review aims to determine the association between SUA and T2DM. It also reflects upon the diabetic complications associated with HUA, and focuses on the health implications of HUA in T2DM patients. Research articles from the year 2005 to 2023 were selected for this review. Articles in English, with abstracts and full texts available, and open accessible were included.

Prevalence of HUA:

According to a systematic review the prevalence of HUA was reported to be 19.01% in T2DM patients.[8] The prevalence of HUA varies in different countries; in India it was found to be 25.8%, 18% in Indonesia, 6% to 25% in China and 10% to 52% in Taiwan. In Uganda, the prevalence of HUA was found to be 38.57%.[3] In a Chinese study the frequency was reported as 35.3%. Another study revealed that the prevalence of HUA ranged from 13.1% to 13.3% in China, 11.9% to 25% in Europe and 11.3% to 47% in USA.[8] In a study

conducted in T2DM patients in Pakistan, HUA was found to be 36.04%, with 47.5% and 52.5% prevalence in females and males respectively.[6]

Primary, secondary and hereditary causes of HUA:

Primary or hereditary causes of HUA include Lesch Nyhann Syndrome and Kelley Seegmiller Syndrome. Secondary causes of HUA include high cell turnover in cancers and myeloproliferative disorders such as lymphomas and leukaemias, tumour lysis syndrome, chemotherapy and radiotherapy, excessive intake of alcohol, fructose and purine rich diet. These account for 10%, while renal insufficiency accounts for 90% of HUA.[9,10]

Role of Uric Acid in Type 2 Diabetes mellitus:

HUA is linked with pancreatic beta (β) cell dysfunction and insulin resistance. The mechanism which is involved in T2DM includes inflammation, oxidative stress and nitric oxide (NO) induced stress of β cells produced by HUA. Glucose transporter 9 (GLUT 9) is considered to be a carrier which is responsible for β cell function and urate transportation.[11] HUA is a major contributing factor in the development of DM and it also contributes independently to diabetic complications.[3]

One of the major complications of diabetes is DKD which comprises of Chronic Renal Failure (CRF) and End-Stage Renal Disease (ESRD). Elevated blood pressure, proteinuria and decrease in estimated Glomerular Filtration Rate (eGFR) are the hallmarks of diabetic nephropathy (DN). The pathogenesis of HUA associated DN involves activation of Renin-Angiotensin-Aldosterone system and oxidative stress. The risk of DN increases with rise in SUA in diabetic patients. SUA not only serves as a potential biomarker but also independently contributes in the development of Chronic Kidney Disease (CKD) and later cardiovascular complications. Therefore it is a predictor of diabetic complications.[12] It has a pathogenic role in their development. These in turn contribute to morbidity and mortality in diabetic patients.[13] A large majority of deaths in diabetic patients is attributed to CRF.[14] Other nephropathies associated with SUA are polycystic kidney disease, immunoglobulin A (Ig A) nephropathy and renal transplantation.

Genetic predisposition of uric acid disturbances:

Several genes encode the uric acid transporters and play a possible role in maintenance of uric acid homeostasis. SLC2A9 (GLUT9) is a transporter gene which if mutated is responsible for derangement of uric acid levels. Epigenetic modification and the role of genetic and environmental factors play a role in SUA levels.[15]

Theories related to HUA and DM:

There are several research studies in terms of uric acid induced DM and elevated SUA levels in DM which are suggestive of a temporal association between HUA and T2DM. There are two central theories that interplay. Firstly SUA secretion is inhibited by insulin resistance and hyperinsulinemia in turn leads to HUA through increased SUA reabsorption. Secondly, HUA leads to oxidative stress and inflammation and decreased levels of NO causing dysfunction of pancreatic β cells. Both are associated with diabetic complications subsequently.[11,16] Some animal studies are also suggestive that insulin secretion is inhibited by HUA.[14]

Pancreatic β cells and insulin resistance:

Through Insulin Receptor Substrate 2/Ak strain transforming or Kinase B protein (IRS2/Akt) signalling pathway in pancreatic β-cells, HUA induces insulin resistance by impairing mitochondrial function. There is also apoptosis of pancreatic β-cells.[17] Insulin contributes in 75% of glucose uptake in skeletal muscles. This is inhibited by HUA in myoblast cell line (C2C12) skeletal muscles.[18] There is a correlation between HUA and adipose induced insulin resistance, which is related to IRS/PI3K/Akt signalling pathway phosphorylation.[19]

Complications of DM related to elevated uric acid:

Oxidative stress:

Excessive production of reactive oxygen species (ROS) is termed as oxidative stress. Elevated levels of uric acid lead to up-regulation of oxidant producing enzymes and thus increased oxidative stress.[20] This leads to vascular inflammation and endothelial dysfunction and cellular damage, and affects insulin gene expression through loss of transcription factors, thus causing decreased secretion of insulin hormone. This eventually results in excessive Xanthine Oxide (XO) production and ROS formation, and diabetes with its complications.[21]

Endothelial dysfunction:

Endothelium releases vasoconstrictors and vasodilators which regulate oxidation, inflammation, vascular tone and thrombosis. Vasoconstrictors include endothelin-1, angiotensin-II and thromboxane A_2, while vasodilators include prostaglandin I_2 and NO. An imbalance between these vasodilators and vasoconstrictors results in endothelial dysfunction.[22] A deficiency of endothelial derived NO results in vascular endothelial dysfunction. This eventually leads to vascular disease and hypertension.[21]

Inflammation:

The expression of CRP, tumour necrosis factor-α (TNFα), interleukin-1β (IL-1β), and interleukin-6 (IL-6) is enhanced by HUA, thus leading to inflammation.[23] The proinflammatory cytokines are released from adipose tissue, pancreatic β-cells, cardiomyocytes, vascular endothelial cells, skeletal muscles, liver, and macrophages. Intracellularly uric acid acts as a pro-oxidant agent while it acts as an antioxidant extra-cellularly.[22] These inflammatory markers are present in much higher urinary concentration in hyperglycaemia as compared to normoglycaemia.[21]

SUA activates several signalling pathways which include mitogen-activated protein kinase (MAPK) pathway. This pathway results in the activation of extracellular signal-regulated kinase (ERK) and p38 group of mitogen-activated protein kinase (p38 MAPK) which release inflammatory cytokines. Another pathway which SUA activates is phosphatidylinositol-3 kinase-Protein kinase B (PI3K)-Akt pathway which also promotes inflammation. Adenosine monophosphate-activated protein kinase (AMPK) pathway which when suppressed by SUA also leads to inflammation.[24]

Renin-Angiotensin-Aldosterone System:
Elevated SUA levels lead to activation of the Renin-Angiotensin-Aldosterone System which results in renal and cardiovascular complications because of increased glomerular pressure and vascular endothelial dysfunction.[23]

Thrombus formation:
Uric acid also gives rise to thrombus formation in vessels through platelet adhesion and aggregation.[23]

Macroangiopathy in DM:
The macrovascular complications in DM include peripheral arterial disease, ischemic heart disease, peripheral vascular disease and cerebrovascular disease.[25] This is caused as a result of hyperglycaemia, altered metabolism and insulin resistance. Macroangiopathy is characterised by angiogenesis, capillary permeability and oedema which give rise to atherosclerosis and rupture of plaques.[26]

Association of uric acid with other diseases:
HUA is also found to be associated with other clinical entities including ischemic heart disease, stroke, hypertension, renal insufficiency, urolithiasis, primary gout, inflammatory arthritis, nephropathy and metabolic syndrome.[27] Neurologic disorders include Alzheimer's disease, Parkinson's disease and Multiple sclerosis. Other conditions also associated with HUA include vitamin D deficiency, cardiac failure, dyslipidemia and obesity. There is involvement of organ systems with the interplay between cell signalling and metabolism.[28] A correlation of HUA also exists with other comorbidities of DM which include neuropathy, retinopathy, nephropathy and Non-alcoholic Fatty Liver Disease (NAFLD). Neovascularization, exudates and microaneurysm are suggestive of retinopathy of both proliferative and non-proliferative types. Diabetic nephropathy determined by eGFR, and microalbuminuria later result in CKD. Diabetic neuropathy included sensory deficit, numbness, tingling and peripheral arterial disease.[29] Hypouricemia is found to be associated with chronic obstructive pulmonary disease (COPD), Parkinson's and Alzheimer's disease. Both fasting blood sugar (FBS) and SUA are associated with loss of renal function.[30] Diabetic complications are also classified as macrovascular (cerebrovascular disease, peripheral arterial disease and coronary heart disease) and microvascular (retinopathy, nephropathy, and neuropathy) respectively. Other life threatening emergencies include diabetic ketoacidosis (DKA) and hyperosmolar non-ketotic coma for diabetes mellitus Type-1 and Type-2 respectively. Atrial fibrillation and severe cardiac failure are also considered as diabetic complications of HUA.[31]

Theoretical application of research to clinical practice:
The recommendations regarding dosage, titration, and effective target SUA levels take into consideration the adverse effects and weigh the potential benefits and risks of initiating an HUA lowering therapy in asymptomatic HUA patients. This decision-making rests with the consulting physicians. Some research studies are suggestive of treatment only in the presence of severity of HUA and symptoms. There are also certain medications which when discontinued result in normal SUA levels such as Thiazide and loop diuretics.[32] A study suggested that SUA lowering drugs in diabetics with DN resulted in controlled glycaemic levels.[14]

Guidelines for management of hyperuricemia:
According to the 3rd edition of Japanese Guidelines on the Management of Asymptomatic HUA, treatment of asymptomatic HUA or HUA with complications is not recommended through pharmacological therapy.[33] There are different criteria for the management of HUA, and research is suggestive of treatment following only urate nephrolithiasis, arthropathy, tophi, and recurrent flares of gout and in patients with several comorbidities. However, guidelines recommend that asymptomatic HUA should remain untreated.[24] Thus the treatment of asymptomatic HUA through urate lowering drugs is still inconclusive. The recommendations of the Japanese Society of Gout are initiation of therapy at SUA levels of 8 mg/dL; while asymptomatic HUA should remain untreated according to the recommendations of the 2020 American Society of Rheumatology Guidelines.[34]

Treatment of HUA:
Hypouricemic drugs aim at lowering the SUA levels. There are two categories of drugs used for the treatment of HUA. These are called uricostatic and uricosuric drugs. The former category includes allopurinol and febuxostat and the later includes benzbromarone, probeneceid and sulphinpyrazone. Uricostatic drugs function by decreasing production of uric acid while uricostatic drugs increase excretion of uric acid.[35] Other classes of drugs include recombinant uricases. Along with xanthine oxidase inhibitors, Lesinurad is also being used for the treatment of HUA.[4] Uric acid lowering drugs such as xanthine oxidase inhibitors used for the treatment of asymptomatic HUA also improve insulin resistance and systemic inflammation. This is because it is hypothesized that SUA lowers NO production and

causes down regulation of insulin sensitisers, thus contributing to insulin resistance.[36]

METHOD

Literature search was done in PubMed and Google Scholar. Initial search revealed 17,500 results of published articles using the terms association of SUA and T2DM, out of which 6,930 (39.6%) articles were found relevant, 276 (4%) were selected for evaluation of title and abstract while 15 (5.4%) were assessed for full-text and subsequently these 15 articles fulfilling the required criteria of original articles were included. Systematic and narrative reviews were excluded from the analysis. (Figure-1)

RESULTS

Literature did not reveal many review articles based on association between SUA and HbA1c in T2DM patients. Literature search conducted from PubMed and Google Scholar resulted in short-listing of 15 articles which revealed the themes of correlation and association of SUA with T2DM. Out of these 15 articles, 8 were consistent with a negative association while 6 were suggestive of a positive association between SUA and T2DM. However in these 6 studies, 1 suggested a partial association of SUA with T2DM, 1 was found prevalent in the female gender only and one explained negative association of SUA with uncontrolled T2DM. One study revealed association of SUA with both hypouricemia and HUA. Thus, SUA was found to be negatively associated with HbA1c and T2DM. These findings are summarized in Table-1.

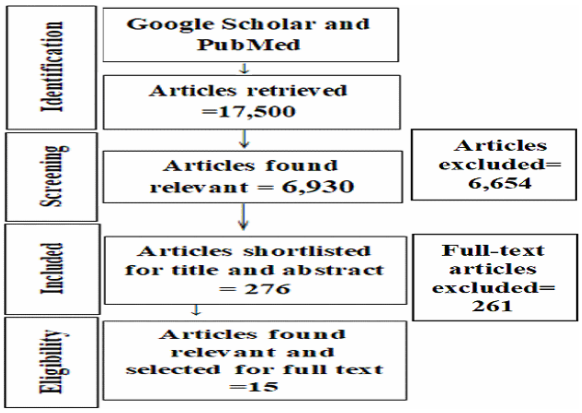

Figure-1: Flowchart based on literature search on association between SUA and T2DM

Table-1: Summary of selected research articles based on association of SUA with T2DM

No.	Authors	References	Results
1.	Yuan *et al*	37	SUA levels decreased with higher glycaemic levels in T2DM patients in comparison with normoglycaemic and impaired glucose intolerance individuals
2.	Hidayat *et al*	38	A negative correlation between SUA and HbA1c and thus T2DM
3.	Xue *et al*	39	Levels of SUA were found to be lower in newly diagnosed or already diagnosed T2DM patients
4.	F. Wei *et al*	40	SUA was found to be inversely related with HbA1c and FBS in T2DM patients
5.	Neupane *et al*	41	SUA and HbA1c were positively associated
6.	Cui *et al*	42	The correlation between SUA and HbA1c is negative in newly diagnosed T2DM patients
7.	Hussain *et al*	43	There was a significant association between SUA and T2DM
8.	Kawamoto *et al*	44	SUA and HbA1c were significantly associated with the female gender
9.	Donkeng *et al*	45	A positive correlation was found between HUA and HbA1c in both controlled and uncontrolled T2DM; while no association was found between HUA and uncontrolled T2DM. HUA was found in female patients >45 years of age
10.	Samin *et al*	46	There was a significant association of SUA with T2DM, however HbA1c was not taken into consideration
11.	Y. Wei *et al*	47	There was an inverse relationship between SUA and HbA1cin T2DM patients
12.	Dhungana *et al*	48	Negative correlation between SUA and HbA1c
13.	Singh *et al*	49	In newly diagnosed patients both hypouricemia and hyperuricemia was found prevalent
14.	Ali *et al*	6	The relationship between SUA and T2DM was found to be linear
15.	Beniwal *et al*	7	No association between SUA and HbA1c

Factors associated with variation in SUA levels:

There are several factors responsible for variation in the prevalence of HUA; these include environmental, geographic, and genetic variations, as well as food preferences and life styles. It is also dependent on the cut-off values taken into consideration for normal SUA values for both the genders separately. Thus patients with HUA are more prone to develop complications in T2DM as compared to normouricemic patients.[3] There are also other modifiable risk factors which could possibly affect SUA levels such as diuretics, alcohol, Dietary Approaches to Stop Hypertension (DASH) style diet and Body Mass Index (BMI). The DASH-diet is based on less sodium intake, beverages, red meat, and higher intake of legumes, nuts, fruits and vegetables, whole grains and low dairy products. The variance in SUA is also based on common genome wide genes. However, several times it is observed that prevalence, exposure or causal effect and variance do not correspond.[50] HUA is also significantly associated with dietary factors such as consumption of seafood and red meat however it was not associated with total protein intake. An inversely related association with cheese, eggs, skimmed milk and non-citrus fruits was also found. However some studies are suggestive of genome wide single nucleotide variance contributing to 23.9% variation in SUA levels. Other factors contributing to renal clearance of SUA are genetic and clinical variants. It also varies with respect to different population distribution. Fractional Excretion of Uric Acid (FEUA) may also have a role to play in the

renal control of SUA excretion related to population specific differences in individuals.[51]

There is a gender variation with relatively lower SUA levels in females as compared to males. This is because of the differences in renal handling of urate in either gender and the uricosuric acid effects of the reproductive hormone oestrogen in the female gender. Therefore the mechanisms involved include higher uric acid fractional excretion and lower uric acid tubular postsecretory reabsorption. Thus it is suggested that a higher level of oestrogen caused higher clearance of SUA levels. A study conducted in premenopausal and postmenopausal females revealed that the SUA levels were found to be higher in postmenopausal women as compared to premenopausal women.[52]

According to a study conducted in China it was revealed that SUA levels increased when HbA1c levels were >5.7%; however they decreased with a further increase in HbA1c levels. Thus SUA levels were moderately elevated with moderate rise in HbA1c. The mechanism by which SUA results in the inhibition of insulin signalling is the recruitment of ectonucleotide pyrophosphatase/phosphodiesterase-1 (ENPP1). SUA genes have a role to interplay in the differences in either gender; SUA in women is lowered by the gene SLC2A9, while in men by ABCG2.[41]

A study revealed that SUA was inversely related with HbA1c but only when insulin levels were high, however there was no correlation when the levels were low. This could possibly be because hexose monophosphate shunt increases both biogenesis and uricogenesis under the influence of hyperinsulinemia. Insulin also promotes reabsorption of SUA in the proximal convoluted tubule. Thus both SUA and HbA1c levels are regulated by insulin.[42] There is a variation and fluctuation in SUA levels with respect to glycaemic control and gender. Studies are suggestive of a bell-shaped relationship between SUA and HbA1c which explain that the levels of SUA are elevated moderately when HbA1c levels are <7% and decline when >7%.[38] Several other reasons have been proposed for lower SUA levels in diabetics; these are low calorie intake, increase in extracellular volume, glycosuria, hyperfiltration and osmotic diuresis.[14] Glycosuria further results in decreased reabsorption of sodium ions in the proximal convoluted tubule, thus further decreasing tubular SUA reabsorption.[48]

CONCLUSION

Several relationships have been developed between the association of HUA and T2DM, which are bi-directionally related. Both are considered as metabolic disorders and either T2DM leads to the development of HUA or HUA is a causative factor in the incidence of T2DM. Our findings are suggestive that SUA and T2DM were negatively associated. Some studies are suggestive of a positive association between SUA and T2DM and development of complications in T2DM. SUA levels have a pathogenic role in the development of diabetic complications of nephropathy. The levels of SUA could possibly be investigated for early detection of diabetic complications, specifically in T2DM patients with moderately controlled glycaemic control. SUA and T2DM were negatively associated in 8 research studies and positively associated in 3 studies. However, one study revealed that SUA was associated with both hypouricemia and HUA.

DISCLOSURE

A part of this narrative review article is based on the M. Phil. (Physiology) thesis submitted to Bahria University Health Sciences Campus, Karachi as a requirement for postgraduate degree.

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Address for Correspondence:

Dr Fatima Zehra, Department of Medical Education, Bahria University, Health Sciences Campus, Karachi, Pakistan.
Cell: +92 332 3098338
Email: fatimazehra0508@gmail.com

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