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EDITORIAL

MONKEYPOX —A LOOMING HEALTH CRISIS FOR THE WORLD

Tehseen Iqbal
RYK Medical & Dental College, Rahim Yar Khan, Pakistan

Monkeypox is similar to Small Pox but it is less severe and less infectious than the small pox. Smallpox vaccination was approximately 85% protective against monkeypox. Ninety-two confirmed cases and twenty-eight suspected cases of Monkeyvirus were reported to World Health Organization between 13 to 21 May 2022 in 12 non-endemic countries in Europe, the United States, and the United Kingdom. Monkeypox is still a serious illness because of its complications, from the effects of sepsis and encephalitis to blindness from eye infections; nearly one in ten people infected are at risk of fatal complications, especially among young children. There is no diagnostic facility available in Pakistan. Health professionals should rely on their clinical skills to diagnose monkeypox. Flu-like symptoms are common initially, ranging from fever and headache to shortness of breath. One to 10 days later, a rash can appear on the extremities, head or torso that eventually turns into blisters filled with pus. Monkeypox produces smallpox-like skin lesions. Macules leading to Papules, Vesicles, Pustules, Scabs, Rash resolved in 14 to 21 days. An antiviral agent known as Tecovirimat is licensed by the European Medical Association (EMA) for monkeypox in 2022.

Keywords: Monkeypox, non-endemic spread, Tecovirimat

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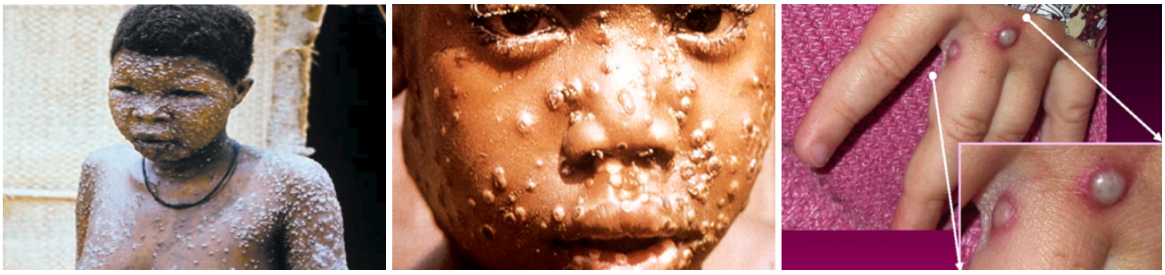
Like COVID-19, monkeypox is a viral disease but it is threatening because it is spreading outside its endemic areas. Monkeypox is similar to smallpox but it is less severe and less infectious than the smallpox. In light of monkeypox cases spreading across Europe, the United States, and the United Kingdom, Pakistan's top health body Monday, May 23, 2022, issued an alert regarding the virus. National Institute of Health (NIH), Pakistan puts federal and provincial health officials on high alert. Instructions have been issued to monitor passengers at airports and other entrances. Medical staff is advised to be careful around monkeypox patients.[1]

Historical data have indicated that smallpox vaccination with vaccinia virus (another orthopoxvirus) was approximately 85% protective against monkeypox. However, following the eradication of smallpox in 1980, routine vaccination against smallpox was no longer indicated, and it has now been four decades since any orthopoxvirus vaccination program.[2]

Twelve non-endemic countries reported to WHO (between 13 to 21 May 2022) 92 confirmed cases and 28 suspected cases of monkeyvirus. Monkeypox is a viral zoonosis (a virus transmitted to humans from animals) with symptoms very similar to those seen in the past in smallpox patients, although it is clinically less severe. It is caused by the monkeypox virus which belongs to the *orthopoxvirus* genus of the *Poxviridae* family. There are two clades of monkeypox virus: the West African clade and the Congo Basin (Central African) clade. The name monkeypox originates from the initial discovery of the virus in monkeys in a Danish laboratory in 1958. The first human case was identified in a child in the Democratic Republic of Congo in 1970. Monkeypox is usually self-limiting but may be severe in some individuals such as children, pregnant women, or persons with immune suppression due to other health conditions. Human infections with the West African clade appear to cause less severe disease compared to the Congo Basin clade, with a case fatality rate of 3.6% compared to 10.6% for the Congo Basin clade.[3]

Nonetheless, monkeypox is still seen as a serious illness that carries a risk of ongoing complications, from the effects of sepsis and encephalitis to blindness from eye infections. Without medical treatment or vaccination, nearly one in ten people infected are at risk of fatal complications, especially among young children. Compared with the horrors of smallpox, which at its peak claimed nearly one out of every three infected, monkeypox might not seem that bad. But if we've learned anything from the COVID-19 pandemic, it's better to be safe than sorry when it comes to potentially deadly viruses.[4]

A definitive diagnosis is accomplished via polymerase chain reaction (PCR) testing of skin lesions or fluid. Although Federal Ministry of Health and National Institute of Health refute reports of suspected cases of monkeypox in Pakistan, the officials of the federal health ministry said that currently there is no facility of diagnostic tests for the virus is present in the country.[5] So, health professionals should rely on their clinical skills to diagnose monkeypox cases. History of travel from the endemic areas or from the countries now infected is important. The virus can be transmitted through contact with an infected person or animal or contaminated surfaces. Researchers believe that human-to-human transmission is mostly through inhalation of large respiratory droplets rather than direct contact with bodily fluids or indirect contact through clothes.



Monkeypox Skin Lesions

Typically, the virus enters the body through broken skin, inhalation, or the mucous membranes in the eyes, nose or mouth. After the virus enters the body, it starts to replicate and spread through the body via the bloodstream. Symptoms usually don't appear until one to two weeks after infection. Monkeypox produces smallpox-like skin lesions, but symptoms are usually milder than those of smallpox. Flu-like symptoms are common initially, ranging from fever and headache to shortness of breath. One to 10 days later, a rash can appear on the extremities, head or torso that eventually turns into blisters filled with pus. Overall, symptoms usually last for 2 to 4 weeks, while skin lesions usually scab over in 14 to 21 days.[5] Lymphadenopathy is a distinguishing feature of monkeypox from smallpox. Enanthem –the first lesions to develop are on the tongue and in the mouth. Macules develop after 1−2 days, Papules in next 1−2 days, Vesicles in another 1−2 days, and Pustules in next 5−7 days. Scabs remain for the next 7−14 days and then the Rash is resolved. Pitted scars and/or areas of lighter or darker skin may remain after scabs have fallen off. Once all scabs have fallen off a person is no longer contagious.[7]

According to the World Health Organization, clinical care for monkeypox should be to alleviate symptoms, manage complications, and prevent long-term sequelae. Adequate nutritional status can be maintained throughout consumption of fluids and healthy food. An antiviral agent known as Tecovirimat that was developed for smallpox was licensed by the European Medical Association (EMA) for monkeypox in 2022. However, Tecovirimat is not yet widely available. A monkeypox outbreak can be controlled with the help of smallpox vaccine, Cidofovir, Tecovirimat (ST-246) and Vaccinia Immune Globulin (VIG).[8] Probably none of these treatments are available in Pakistan.

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ORIGINAL ARTICLE

ROLE OF ASCORBIC ACID ON ALTERED LEVELS OF TESTOSTERONE DURING CHEMOTHERAPY

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Background: Improvement in quality of life of cancer survivors has become a significant healthcare dilemma. Despite having important part in the support of spermatogenesis, few studies are conducted on Leydig cells secretion, i.e., testosterone which has an important role in fertility The objective of this study was to demonstrate altered serum testosterone levels by anthracycline on mice and to study changes observed in serum level in DOX affected mice with co-administration of antioxidant ascorbic acid. **Methodology:** Male mice aged five weeks were used. The animals were divided into 3 groups of 10 mice each. Animals in Group A were given normal saline intraperitoneally (IP). Group B mice were given DOX IP alone. In Group C, DOX and ascorbic acid were given orally. Blood samples were taken through cardiac puncture. Sera from centrifuged blood were tested for testosterone using ELISA kit. **Results:** Serum testosterone levels were 1.61±1.77, 6.22±4.78, and 1.98±0.45 ng /ml in Group A, B and C respectively. The administration of DOX induced significant increase (p<0.001) in the serum testosterone level. However co-administration of Ascorbic acid with DOX significantly reduced (p<0.001) the serum testosterone level. **Conclusion:** Ascorbic acid has a role against DOX induced altered hormonal levels.

Keywords: Doxorubicin, Ascorbic acid, Mice, Antioxidant, Testosterone

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INTRODUCTION

Usage of chemotherapeutic agents is significantly growing owing to increased number of malignancies globally.[1] Despite their side effects on healthy proliferating cells, their use has become a cornerstone in the process of treatment and has proven to be mitigating effect on cancer complications.[2] Doxorubicin is a widely used chemotherapeutic drug that belongs to anthracycline family. From the very beginning after its induction in cancer guideline therapy, it has been approved and used as a first-line treatment of various cancers both in adults or children.[3] Its mechanism of action is chiefly by inhibiting enzymes crucial for DNA replication.[4] In addition, it produces free radicals that overcame the antioxidant capacity of cells resulting in cytotoxicity.[5]

Experiments have supplied enough evidences regarding doxorubicin's adverse effect on testicular tissue through inducing oxidative stress and apoptosis.[6] Its intercalation into strands of DNA results in double-strand breaks and causes cell death[7,8] in dividing testicular germ cells[9]. The very high vulnerability of sperms and testes to doxorubicin induced damage might be due to a weak anti-oxidant defence mechanism in testicular tissue.[10] Earlier studies have demonstrated pivotal role of antioxidants in improving the quality of the different testicular parameters in infertile males.[11] Keeping this in mind, various antioxidants have been administered to save the testes from doxorubicin injury.[12,13]

Ascorbic acid is from the free radical scavenging group of antioxidants.[14,15] Ascorbic acid has been observed to decline the chances of development of breast cancer.[16] As one of the strong antioxidant, ascorbic acid seems to produce hurdles in DOX producing free radical formation.[17] Marked deficiency in the levels of antioxidants including ascorbic acid was seen in the therapy of DOX in breast cancer. It increases the tendency of healthy tissues to radical damage, proving the call to use antioxidants along with anticancer therapy.[18] The objective of this study was to see the levels of testosterone in DOX affected animals with co-administration of ascorbic acid.

MATERIAL AND METHODS

This study was conducted at the Institute of Basic Medical Sciences, Dow University of Health Sciences (DUHS) Karachi with the approval from the Local Research Committee, Institutional Review Board (IRB), Funding Committee of Dow University and Board of Advance Studies Review (BASR) DUHS Karachi.

Only health thirty male mice of NMRI Strain which were 5 weeks old and caged in animal house of, under normal circadian rhythm. The animals were divided into 3 groups of 10 each. Group A (Control Group) with 10 male mice received 1 ml of normal saline IP on 6th, 8th and 10th day of the study. In Group B

(DOX group) , animals had DOX in dosage of 0.003 mg/g or 0.003 mg in 0.03 ml/gm body weight IP[6] up to 3 doses on 6th, 8th and 10th day of study (total cumulated dose 0.009 mg/gm). Group C (DOX+ascorbic acid group) animals received DOX in a dose of 0.003 mg/gm or 0.003 mg in 0.03 ml/gm body weight IP[6] and ascorbic acid in dose of 0.5 mg/gm or 0.5 mg in 0.01 ml/gm body weight per oral.[19] DOX on 6th, 8th and 10th day of experiment, and ascorbic acid daily.

After exposure, blood samples were collected via cardiac puncture from each mouse. They were run in serum testosterone rat/mouse ELISA kit by Demeditec DEV9911 Germany in Dow Diagnostic Researcj and Reference Laboratory, DUHS.

One-way Analysis Of Variance (ANOVA) was used and then Tukey-Multiple Comparisons Post Hoc test was applied to check the pair-wise comparison at 5% level of significance (95% confidence interval CI).

RESULTS

The Mean±SD serum testosterone in the control group A was 1.61±1.77 ng/ml and in DOX group B it was 6.22±4.78 ng/ml. When the means of control and DOX groups were compared, it showed the *p*-value 0.005 at 95% Confidence interval. A significant increase was observed in the serum testosterone level in DOX group.

Comparison between B and C, Mean±SD observed for serum testosterone group B was 6.22±4.78 ng/ml whereas the Mean±SD observed in DOX+ ascorbic acid group was 1.98±0.45 ng/ml. The *p*-value for this comparison was 0.009 at 95% CI using Tukey-Multiple Comparisons Post Hoc test. A significant decrease was observed in the serum testosterone level in DOX+ascorbic acid group.

Comparison between the A and C, the Mean±SD observed for serum testosterone in control group was 1.61±1.77 ng/ml whereas in DOX+ascorbic acid treated group the Mean±SD was found to be 1.98±0.45 ng/ml. An insignificant increase was observed in the serum testosterone level in DOX+ ascorbic acid group. The *p*-value observed for this comparison was 0.958 at 95% CI (Table-1).

Table-1: Comparison of mean serum testosterone levels in groups (ng/ml)

Groups	Mean±SD	95% Confidence Interval		Tukey HSD Post Hoc multiple comparisons test	
		Lower Bound	Upper Bound		
Control (A)	1.61±1.77	-7.88	-1.33	A *vs* B	*p*=0.005*
DOX (B)	6.22±4.78	0.9582	7.5218	B *vs* C	*p*=0.009*
DOX+ascorbic acid (C)	1.98±0.45	-2.9118	3.6518	C *vs* A	*p*=0.958

*Significant

DISCUSSION

The protective effects of ascorbic acid over other organs of the body have already been established. It seemed to have nullifying effect against the adverse actions of various substances including gamma irradiation, cadmium, mercury, other anthracyclines and organ phosphorous.[20] Role of ascorbic acid in diseases is evident from various studies. Its beneficial part to health may be attributed to its immunomodulator activity along with antioxidant property.[21] The potential of the healthy nutritional environment demonstrated on male reproductive tract in animals.[22] Animals with deficiency of vitamin C show disturbed spermatogenesis process leading to hormonal imbalance.[10] The significant change was observed between DOX treated and DOX+ ascorbic acid treated groups and insignificant change between control and DOX+ascorbic acid groups was noticed.

Alteration in Leydig cells could lead to imbalance in hormones. Increased serum testosterone level in our study was in contrast with the findings by Ward *et al*[23], who reported serum testosterone levels were not significantly affected by this chemotherapeutic treatment. Another study showed that doxorubicin caused a decrease in free testosterone and FSH levels, the later significantly increased as a result of pre-treatment with rutin and/or hesperidin[24] which was in contrast to both our and another study[23]. In another study vitamin C had significant effect on testosterone level and quality of sperm in gentamicin-induced Wistar rats[25]. The germinal cell, because of active division of mitosis and meiosis seems to be more susceptible to chemotherapy. It can be assumed that secretion of testosterone by Leydig cells remains active after treatment with chemotherapeutic agent[26], but need to be further evaluated.

CONCLUSION

Change in level of serum testosterone is indicating increase number of Leydig cells by chemotherapeutic agent. At the same time alteration in levels after receiving ascorbic acid suggest that its effect on chemotherapeutic agent.

RECOMMENDATION

Clinical studies must be conducted to determine both the short-term and long-term impact of antioxidants, singly and in combination, on the efficacy of cancer chemotherapy and on the development of chemotherapy induced side effects especially their effect on hormonal levels and the mechanism of action through which they impact on the levels of hormone.

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ORIGINAL ARTICLE

COMPARISON OF DIET INDUCED METABOLIC SYNDROME WITH ARTIFICIALLY INDUCED METABOLIC SYNDROME IN A RAT MODEL

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Background: Metabolic syndrome is an emerging health problem. The diets rich in fats and refined carbohydrates and monosodium glutamate are considered major risk factors for this emerging epidemic. The aim of this study was to determine whether high fat high carbohydrate (HFHC) diet, Monosodium glutamate (MSG) diet or their combination is faster/more potent inducer of MS. **Methods:** Twenty male Sprague Dawley rats were randomly divided into four groups. Group 1 was given normal rat chow, group 2 was given HFHC diet, group 3 was given MSG in diet and group 4 was given both HFHC and MSG in their diet for 20 weeks. Body weight, blood pressure, lipid profile and glycaemic indices were determined at the end of study. **Results:** After 20 weeks of study, HFHC and MSG groups showed full blown Metabolic syndrome (MS) with development of obesity, hypertension, hyperglycaemia and dyslipidemia. However, group 4 which was given combination diet did not develop features of MS. **Conclusion:** Both HFHC and MSG containing diets when given alone are potent inducers of MS in rat model rather than their combination.

Keywords: Metabolic syndrome, high fat high carbohydrate diet, monosodium glutamate, obesity, hypertension, dyslipidemia, insulin resistance

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INTRODUCTION

Non-communicable diseases (NCD) have become a major cause of morbidity and mortality in today's world in both developed and developing countries. Among these NCDs, Metabolic Syndrome (MS) has emerged as a global epidemic.[1] Metabolic syndrome or syndrome X is a cluster of medical abnormalities characterized by presence of three or more of the following pathologies: hypertension, hyperglycaemia, dyslipidemia, obesity and insulin resistance.[2] Other co-morbidities with MS include pro-thrombotic and pro-inflammatory states, non-alcoholic steatohepatitis, reproductive disorders and certain types of cancer.[3] Patients with MS are at two fold increased risk of developing cardiovascular disease (CVD) and five fold increased risk of type 2 diabetes mellitus compared to normal people.[4]

The prevalence of MS is rapidly increasing worldwide primarily due to sedentary lifestyles and unhealthy eating habits.[5] Other contributing factors and mechanisms include insulin resistance, adipose tissue dysfunction, chronic inflammation, oxidative stress, circadian disruption, microbiota, genetic factors etc.[6] Strategies aimed at primary prevention are required to ameliorate its further rise as well as reduction of associated morbidity and mortality.[3]

Studies have revealed that consumption of diet and beverages rich in fats and sugars is clearly responsible for increased incidence of MS. Saturated fats and sugars when taken together, become greater risk factor for development of MS than fats or sugars given alone.[7,8] High fat high carbohydrate (HFHC) diet provides excess energy than needed in the body which is then stored in adipose tissue resulting in its expansion and inducing obesity. Increased adiposity is associated with generation of oxidative stress (OS) and local inflammatory changes. OS leads to impaired glucose tolerance and high insulin secretion from pancreas. Increased reactive oxygen species and generation of Angiotensinogen by adipose tissue are responsible for high blood pressure. HFHC diet also induces *de novo* hepatic lipogenesis.[9]

Several epidemiological studies reveal the association of dietary consumption of the chemical Monosodium glutamate (MSG), also called *Ajino moto*, with metabolic disorders such as obesity, hypertension and metabolic syndrome. Its consumption is increasing worldwide as a flavour enhancer, in parallel with the epidemic of metabolic syndrome.[10] It is a sodium salt of the naturally occurring amino acid L-glutamate which elicits a unique taste called 'umami'.[11] It increases the sapidity of food and produces a flavour which cannot be provided by other food additives.[12] It is present in most processed foods, mostly hidden on ingredient labels and listed under other names. Chronic MSG intake may cause glutamate induced damage to arcuate nucleus, disrupting energy regulation axis. It leads to damage to leptin signalling through hypothalamus, causing leptin resistance, overweight and obesity.[13] Chronic administration of MSG induces OS in experimental animals and changes metabolic and endocrine indicators. It induces hypertension due to excessive OS and renal sodium and water retention.[11] It increases hepatic

gluconeogenesis resulting in hyperglycaemia which then causes increased insulin secretion. It also induces insulin resistance via induction of oxidative stress.[14]

Being a rapidly emerging epidemic like disease, researchers are probing more into detailed mechanism of metabolic syndrome and strategies for its prevention. Animal models have a key role in understanding the disease pathophysiology. The aim of this study was to determine whether HFHC diet, MSG diet, or their combination is more potent inducer of MS.

MATERIAL AND METHODS

This was an experimental study. The protocols were approved by the Ethics Committee of PGMI, Lahore, and were conducted in accordance with the guidelines for animal care. The sample size was calculated at 90% power of study and 5% level of confidence. The calculated sample size was five in each group.[15] Twenty male Sprague Dawley rats aged 4–5 weeks, weighing 50–70 g were kept in a controlled temperature of 23±2 °C under natural light and dark cycle, and were fed with standard Rat chow and water *ad libitum* for one week.

After one week of Acclimatization, rats were randomly divided into 4 groups of 5 each with almost equal body weights and were placed on following diets for 20 weeks:

Rats in Group-1 were fed with normal rat chow composed of 48% carbohydrates, 21% protein, 3% fat, 5% fibre, 13% moisture, 8% ash and traces of calcium and phosphorus. Group-2 rats were fed with rat chow in which MSG was mixed in an amount of 5 g MSG/Kg body weight of rats and dose was adjusted per 100 gm diet.[16] Rats in Group-3 were given rat chow in which HFHC diet was mixed. High fat diet consisted of 15% beef tallow (15 gm beef tallow in 100 gm rat chow), 1% cholesterol (1 gm cholesterol in 100 gm rat chow) and 0.5% sodium deoxycholate (0.5 gm in 100 gm rat chow).[17] High fructose diet consisted of 15% fructose (15 gm fructose in 100 ml water) in drinking water.[2] Group-4 rats were fed with rat chow in which both HFHC diet and MSG were mixed.

Body weights of animals were measured weekly using a digital lab weighing scale. Blood pressure was measured at the end of the study on a non-invasive blood pressure measuring system using a pulse transducer connected to PowerLab®. Measurements were considered valid only when 3 consecutive readings did not differ by more than 10 mmHg. After 12 hour overnight fasting, blood samples were taken from cardiac puncture using a disposable syringe with 26 gauge needle under chloroform anaesthesia. After 30 minutes, serum was separated by centrifuge at 3,000 rpm, and stored at -20 °C in serum cups.

Total cholesterol (TC), triglycerides (TGs) and high density lipoproteins (HDL) were measured by Colorimetric method with spectrophotometer. Blood Glucose was measured by Oxidase method with spectrophotometer. Serum Insulin was measured with ELISA with rat insulin kit (Bioassay Technology Laboratory, China), using Sandwich ELISA technique. Serum LDL was calculated with formula: LDL cholesterol=TC-HDL-(TG/5). HOMA-IR was calculated with formula:

HOMA-IR=Fasting Insulin×Fasting Plasma Glucose/405

Data was analysed using SPSS-22. Normality of data was checked by Kolmogorov-Smirnov test. Normally distributed results were presented as Mean±SD. One way ANOVA was applied for comparison among study Groups. Post Hoc Tuckey test was applied to analyse the differences of means among groups, and $p \leq 0.05$ was taken as statistically significant.

RESULTS

At the end of study, intergroup comparison by ANOVA showed significant difference among groups. Results of Post Hoc Tuckey test showed that rats in MSG and HFHC group exhibited a significant weight gain as compared to normal control (NC) group. However, body weight of HFHC+MSG group was significantly less than other groups.

One-way ANOVA showed significant differences of blood pressure among groups. Post Hoc Tuckey test showed that both HFHC and MSG groups showed a significant increase when compared to NC although hypertension was developed more in HFHC than MSG group. There was no significant difference of HFHC+MSG group with NC Group. (Table-1).

Lipid profile changes were variable in different groups at the end of the study, Serum Triglycerides (TG) changes being significant in HFHC group, Serum Cholesterol (TC) changes significant in combination group while Serum HDL was significantly decreased in MSG rats. (Figure-1).

Fasting Hyperglycaemia was clearly developed in MSG and HFHC groups only showing significantly higher values when compared to NC. MSG administration induced higher serum insulin levels both alone as well as in combination diet although results were statistically non-significant while HFHC diet did not induce any remarkable change in insulin level. Results of HOMA-IR indicate that MSG induces higher insulin resistance. (Table-2).

Table-1: Effects of different diets on body weight and BP of rats (n=5 in each)

Groups	Body Weight (gm)	Blood Pressure (mmHg)
NC	262±33.98	99.80±6.61
MSG	335±31.81 *(p=0.006)	124.80±6.18 *(p=0.000)
HFHC	335.60±29.1 *(p=0.005)	133.60±6.84 *(p=0.000)
HFHC+MSG	191.40±18.88 *(p=0.007)	109.80±3.11

*Post Hoc Tuckey test

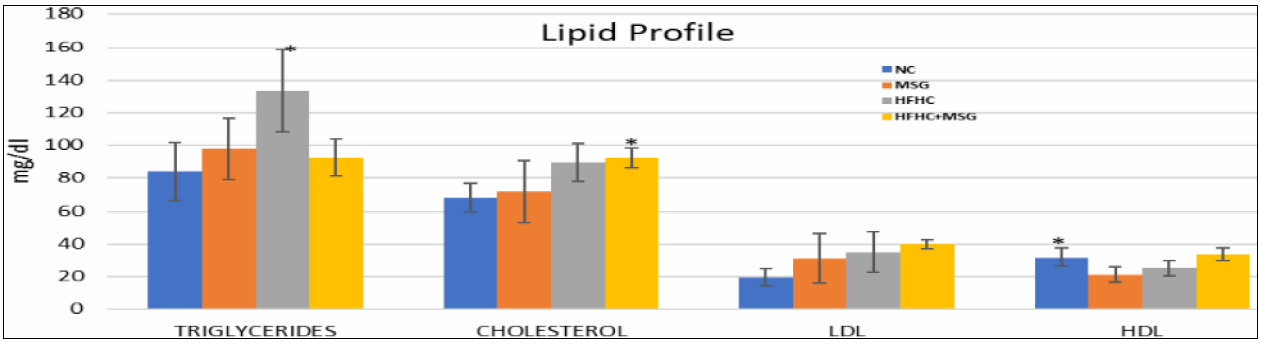

Figure-1: Effects of different diets on Lipid profile of rats

*p<0.05 as compared to NC (Analysed by Post Hoc Tuckey test)

Table-2: Effects of different diets on Glycaemic indices of rats

Groups	Fasting Glucose (mg/dl)	Serum Insulin (mIU/L)	HOMA-IR
NC	84.20±8.58	1.34±1.11	0.27±.23
MSG	157.60±27.75 *(p=0.000)	3.04±2.80	1.17±1.16
HFHC	152.80±8.46 *(p=0.000)	1.78±0.27	0.67±.14
HFHC+MSG	81.80±8.16	3.08±0.31	0.61±.07

*Post Hoc Tuckey test

DISCUSSION

Metabolic syndrome (MS) is a multifactorial condition with alarming rate of prevalence nowadays. To evaluate the pathophysiology of MS in humans, it is crucial to establish appropriate experimental animal models which mimic the disease state in humans.[18] Among rodents, rats and mice are the most common animal models for investigating MS and in rats, Sprague Dawley rats and Wister rats are most commonly used rodents.[19]

Several previous studies have shown the successful induction of MS in animal models with HFHC diet and also with MSG.[7,20] Literature review reveals different experiences in success of induction, duration of induction and parameters affected by MS.[18] we have conducted this study to evaluate whether diet, chemical or a combination of both is potent and faster inducer of MS in rat model.

There was significant development of obesity, hypertension, fasting hyperglycaemia and hyper-triglyceridemia in rats fed HFHC diet as compared to NC rats. Our results confirm the findings of several other researchers.[17,21,22] However, the levels of serum insulin and HOMA-IR although raised from NC rats were statistically not significant which could be due to shorter duration of study. Pathophysiological changes by HFHC diet include excess energy in body resulting in high serum free fatty acid concentration, hypertrophy of adipose tissue, increased oxidative stress and insulin resistance among various other mechanisms.[21,22]

We observed that MS was also successfully induced in MSG rats with development of significant obesity, hypertension, hyperglycaemia, and decreased HDL levels as compared to NC rats. The values of serum insulin and HOMA-IR although higher than NC and HFHC groups but were statistically non-significant which may be due to our rat model in which adult rats were recruited for study, shorter duration of study or insufficient dose of MSG. Our results also coincide with previous human as well as animal studies.[10,13,23–25] Increased oxidative stress, changes in fat and carbohydrate metabolism, sodium and water retention by kidney might be possible mechanisms responsible for development of MS by monosodium glutamate.[11] Another research shows that MSG is linked with obesity, type II diabetes, and the metabolic syndrome as its intake in healthy Chinese adults correlates with the resulting increase in body mass index regardless of energy intake.[23]

On the contrary, rats of combination group (HFHC+MSG) showed reduction in weight gain when compared with NC rats, which might be due to the decreased food intake (noted by leftover food pellets after 24 hrs) and early satiety. Our findings coincide with findings of Onaolapo *et al*.[26] who noted that obesity, hyperglycaemia and dyslipidemia did not develop with this combination diet. These findings are contrary to the findings of some other researchers.[27] This may be due to antagonistic effects of both diets when given in combination as demonstrated by Su *et al*[28] in their research on growing pigs. They observed antagonistic effects of both diets on most fatty acid receptors in gut wall resulting in reduced activity of most fatty acid receptors and resultant altered lipid metabolism. The effects of this combination diet may also be due to less food consumption and early satiety, reduction in lipid peroxidation and possible interaction between the diets altering the expression of lipid metabolizing genes.[26]

CONCLUSION

HFHC diet and diets containing MSG when given individually, are effective inducers of MS in rat model and may be considered for induction of MS in experimental animals. Combination model failed to induce features of MS.

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MS: Concept, Design of work, Proof reading

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ORIGINAL ARTICLE

EFFECT OF RAMADAN FASTING ON POST-EXERCISE HEART RATE RECOVERY AT TWO MINUTES AND BODY COMPOSITION IN HEALTHY MALE ADULTS

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Background: The effects of Ramadan fasting on weight loss are well-known but its effects on heart rate recovery after 2 minutes (HRR2) have not been investigated. The present study aims to observe the effects of Ramadan fasting on body composition variables and post-exercise heart rate recovery after two minutes of rest. **Methods:** A prospective, observational study was conducted from April 2019 to July 2020. Sixty-four healthy male participants aged 26 to 53 years were enrolled on the first day of the fasting in the Islamic month of Ramadan and were followed up for two weeks. Baseline anthropometric measurements and body composition measurements were recorded on day 1 of Ramadan in a fasting state. Peak heart rate and heart rate recovery were recorded immediately after exercise and after two minutes of rest. Blood pressure was also recorded after cessation of exercise. After fourteen days of fasting, anthropometric measurements and aforementioned readings with repeat exercise protocol were recorded. **Results:** Mean BMI, waist circumference and body fat ratio of the study participants reduced significantly after fourteen days of Ramadan fasting. The resting heart rate of the study participants was significantly reduced from 74.5±9.7 to 68.4±10.7 bpm (p<0.001). HRR2 of rest significantly increased from 22.9±11.7 to 30.3±13.7 bpm (p=0.001). None of the smokers showed an improvement in heart rate recovery after two minutes of rest. **Conclusion:** Ramadan fasting resulted in significant improvement in body fat ratio, BMI, waist circumference and heart rate recovery after two minutes of rest in healthy adult males.

Keywords: BMI, body composition, fasting, heart rate recovery

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INTRODUCTION

Fasting has recently generated great interest to combat obesity as a feasible and sustainable strategy as it has shown healthy weight loss with the preservation of muscle mass. There are many variations of fasting which allow normal food and drink consumption in eating windows interposed by periods of energy restriction or fasting to create a state of negative energy balance for weight loss.[1,2] Moreover, accelerated fat loss is associated with several advantages such as improvement in insulin sensitivity, blood sugar, systolic and diastolic blood pressure, total serum cholesterol, inflammatory biomarkers and low-density lipoprotein (LDL).[3,4] There are many approaches for intermittent fasting such as 5:2 modified fasting regimens, alternate day fasting, time-restricted feeding, and religious fasting. One popular version of this approach is the 16:8 fasting protocol with an eating window of eight hours and abstinence from food for 16 hours per day.[5,6]

Fasting is also observed as a religious duty in some cultures and religions. Fasting for 29–30 days is observed by Muslims throughout the world in the lunar month of Ramadan and depending on the season and geographical location of the country, fasting duration can fluctuate from 11 to 22 hours.[7] Though the principle of energy restriction employed by intermittent fasting is not applicable in Islamic fasting, however time-restricted feeding during Ramadan can lead to changes in metabolic and cardiovascular parameters.[8]

The effect of Ramadan fasting on overall health may be assessed by many simple and noninvasive tools such as body mass index (BMI) and body fat ratio (BFR). Post-exercise heart rate recovery (HRR) is another simple and non-invasive method to assess cardiac health by the speed of recovery of heart rate back to normal in a given time after exercise. An abnormal heart rate recovery is defined as less than 12 beats/min (bpm) or less than 22 bpm at 1 and 2 min respectively.[9] Studies have shown that a decrease in body fat ratio by dietary programs leads to improvement in resting heart rate and post-exercise HRR.[10,11] Previous literature has suggested the effect of Ramadan fasting on body composition, biochemical variables, blood pressure and heart rate variability, but to the best of our knowledge, the effect of fasting on HRR2 in healthy volunteers has not been evaluated yet.[12–15] Fasting might prove to be a noninvasive strategy for the improvement of HRR2 and could be employed to improve the overall health of cardiometabolic patients.

The objective of our study was to investigate the effect of Ramadan fasting on weight loss, fat loss and post-exercise heart rate recovery as a non-invasive indicator of cardiovascular health.

METHODOLOGY

This was a prospective observational study and carried out in the lunar month of Ramadan. Ethical approval was sought from Institutional Review Board and Ethics Committee (IRB#903-178-2017) and complied with the ethical standards laid down by the Helsinki declaration of 1964 and its later amendments. Sixty-four faculty and staff male members from our university who fasted in the month of Ramadan volunteered for this study. Written informed consent was obtained from all study participants. Participants with hypertension, diabetes mellitus, cardiovascular or respiratory disorders or any other disorder susceptible to sudden weight loss or gain in body weight in the past three months were excluded from the study on initial interviews. The participants were enrolled on the first day of the fasting lunar month and were followed up for two weeks after recording baseline data. We did not recruit any female participants in this study as Muslim females do not fast during the menstrual phase of the menstrual cycle and there was a possibility of many female participants discontinuing fasting during the study period.

We took baseline anthropometric measurements of every male participant such as weight, height, waist circumference and BMI on the first day of fasting in fasting state. Body composition measurements such as body fat, lean muscle mass and weight of each participant were recorded by a leg-to-leg bioelectrical impedance commercial glass body composition analyzer (Beurer living glass diagnostic scale BG-42). Height was measured with the help of a vertical stadiometer. BMI was calculated using the formula of body weight (Kg)/height $(m)^2$. Participants were categorized into three groups: 18.5–22.9 Kg/m^2 (normal weight), 23–27.5 Kg/m^2 (overweight) and ≥27.5 Kg/m^2 (obese) according to WHO recommended BMI cut-off points for Asians.[16] Waist circumference was measured in Cm to the nearest decimal by using a tape measure.

Systolic and diastolic blood pressure, resting heart rate was measured before exercise. Fitbit Charge 3 device was used for continuous monitoring of heart rate before and after exercise. Details of the exercise protocol have been mentioned in our previously published findings from the same study project in which we have reported the association of HRR2 with body composition variables.[17] The participants were asked to stop walking and take a rest in case they experienced breathlessness, palpitations, leg cramps and dizziness. The peak heart rate of participants was recorded immediately after exercise and after two minutes. Blood pressure was also recorded after cessation of exercise. HRR2 was calculated by measuring the difference between the peak heart rate immediately post-exercise and after resting for two minutes. A difference of less than 22 bpm after two minutes was considered as an impaired heart rate recovery.[9]

According to the Islamic calendar based on the lunar cycle average duration of fasting was 16 hours per day in Ramadan in Pakistan in the year 2019. Subjects consumed food without calorie restriction in the eating window of non-fasting eight hours per day. After fourteen days of fasting weight, height, waist circumference, BMI and body compositions were measured again. The exercise protocol was repeated and all the readings of blood pressure, resting heart rate, peak heart rate and HRR2 were recorded.

Collected data were analyzed using Statistical Package for Social Sciences (SPSS-23) program. Shapiro-Wilk test was applied to data for normality. Students' *t*-test and Wilcoxon signed-rank test were applied for comparison between different groups for normally distributed data and non normally distributed data respectively. Continuous variables were specified in Mean±SD. Categorical variables were described as frequencies, percentages and computed by Chi-square and Fisher's exact test considering $p \leq 0.05$ as statistically significant.

RESULTS

All the recruited participants completed the fasting for fourteen days and there were no dropouts. The mean age of the participants was 35.5±6.6 years. There was a statistically significant decrease in weight, BMI, waist circumference, body fat ratio, blood pressure, and resting heart rate. There was a statistically significant increase in HRR2 after two weeks of fasting. Effects of fasting on baseline parameters and pre and post-exercise variables are shown in Table-1.

There was a significant increase in the HRR2 of all participants but the recovery was more pronounced in participants with BMI <23. Improvement in HRR2 in participants with body fat ratio <25 and moderate physical activity was statistically significant. Changes in heart rate recovery after fourteen days of fasting according to baseline body composition variables and level of physical activity are summarized in Table-2.

Body fat ratio <25 and non-smokers showed a significant association with the increase of ≥20 bpm in HRR2. Association of HRR2 less and more than 20 beats per minute with body fat ratio, BMI, smoking and physical activity are shown in Table-3.

Table-1: Bodyweight, body composition variables, blood pressure, HRR on 1st day and 14th day of fasting

Variables	1st day of fasting (Mean±SD)	14th day of fasting (Mean±SD)	Percent change from baseline	*p*
Weight (Kg)	74.5±11.5	73.2±11.3	-1.7	<0.001*
BMI (Kg/m^2)	25.4±3.9	24.9±3.8	-1.9	<0.001*
†Waist circumference (Cm)	94.4±10.4	91.1±8.2	-3.5	<0.001*
†Body fat (%)	26.1±7.1	22.7±6.4	-13	<0.001*
Systolic BP (mmHg)	121.6±12.3	116.3±6.2	-4.4	0.001*
†Diastolic BP (mmHg)	83.6±8.7	79.1±8.1	-5.4	<0.001*
Mean BP (mmHg)	102.6±9.6	97.7±6.1	-4.8	<0.001*
†Resting HR (bpm)	74.5±9.7	68.4±10.7	-8.2	<0.001*
Peak HR after exercise (bpm)	98.9±10.0	98.2±11.8	-0.7	0.62
†HR after 2 mins of rest (bpm)	76.0±12.87	67.88±13.49	-10.68	< 0.001*
HRR2 (bpm)	22.9±11.7	30.3 ± 13.7	+32.3	0.001*

BMI: Body Mass Index, BP: Blood Pressure, HR: Heart Rate, HRR2: Heart Rate Recovery after two minutes, *Significant, †Wilcoxon signed-rank test

Table-2: Changes in HRR2 after fourteen days of fasting according to body composition variables and physical activity

Variables	Participants n (%)	HRR on 1st day of fasting (Mean±SD)	HRR on the 14th day of fasting (Mean±SD)	Percent change from baseline	*p*
BMI <23	22 (34.4)	20.7±11.0	30.7±12.2	48.3	0.008*
BMI ≥23	42 (65.6)	24.1±12.0	30.1±14.5	24.9	0.026*
BMI <25	30 (46.9)	20.6±12.2	30.7±11.1	49.0	0.002*
BMI ≥25	34 (53.1)	25±10.9	29.9±15.7	19.6	0.096
Fat <25†	22 (34.4)	19.5±11.0	33.7±10.7	72.8	<0.001*
Fat ≥25†	42 (65.6)	24.8±11.7	28.5±14.8	14.9	0.155
Sedentary lifestyle	36 (56.3)	22.6±10.9	27.6±14.6	22.1	0.084
Moderate physical activity	18 (28.1)	24.8±14.8	34.6 ±9.9	39.5	0.004*
Regular physical activity	10 (15.6)	21.0±8.0	32.6 ±14.9	55.2	0.119

*p<0.05, †Wilcoxon signed-rank test

Table-3: Increase in HRR2<20 bpm and ≥20 bpm in participants with different BMI, body fat ratio, smoking and physical activity status

Variables	Increase in HRR2 <20 bpm (n=46), n (%)	Increase in HRR2 ≥20 bpm (n=18), n (%)	*p*
BMI <23	14 (21.9)	8 (12.5)	0.289
BMI ≥23	32 (50)	10 (15.6)	
BMI <25	20 (31.3)	10 (15.6)	0.384
BMI ≥25	26 (40.6)	8 (12.5)	
Body fat ratio <25	12 (18.8)	10 (15.6)	0.026*
Body fat ratio ≥25	34 (53.1)	8 (12.5)	
Non smokers†	36 (56.3)	18 (28.1)	0.031*
Smokers	10 (15.6)	0	
Sedentary lifestyle	26 (40.6)	10 (15.6)	
Moderate physical activity	14 (21.9)	4 (6.3)	0.590
Active lifestyle	6 (9.4)	4 (6.3)	

*p<0.05, †Fisher's exact test

DISCUSSION

Intermittent fasting has shown success as a potential strategy for weight loss and fat reduction.[1] Several trials to evaluate the effects of fasting in humans have shown promising results as fasting preserves muscle mass and has an overall positive effect on body composition variables and heart rate variability.[5,14] In this study we investigated the effect of Ramadan fasting on body composition variables and post-exercise heart rate recovery as a non-invasive indicator of cardiovascular health. Overall, Ramadan fasting had a significant impact on the mean BMI, waist circumference, body fat ratio and HRR2 of our study participants. Heart rate recovery after two minutes of rest significantly increased from 22.9±11.7 to 30.3±13.7 bpm (p=0.001).

The results of our study showed that fasting greatly helped in weight loss and reduction in body fat and resulted in a significant increase in heart rate recovery in most of the participants without any attempt to influence physical activity and diet patterns. We observed a significant decrease in the mean weight, BMI, body fat ratio and waist circumference of all study participants who fasted for fourteen days. Our findings are in agreement with previous similar studies which have reported significant weight loss and reduction in body fat ratio and waist circumference after few days of fasting.[18,19]

There was significant improvement in cardiovascular variables including systolic blood pressure, diastolic blood pressure, resting heart rate and

HRR2. Our findings are in contrast to a recent study conducted in Turkey to observe the effect of Ramadan fasting which did not show significant improvement in resting heart rate and blood pressure.[18] Similarly a study conducted in Ajman, UAE also reported a decrease in weight and BMI but did not show significant changes in systolic and diastolic blood pressures which could be due to the recruitment of patients with metabolic syndrome.[20] However, a recent metaanalysis of 70 studies measuring the effect of Ramadan fasting reported consistent albeit transient reduction in weight and body composition.[19]

To our knowledge, the effect of fasting on resting heart rate and heart rate recovery has not been explored yet in healthy volunteers or any patient population. Various studies have explored the contribution of different predictors in abnormal heart rate recovery; a study conducted by Panzer *et al*[21] established the significant association of impaired fasting plasma glucose levels with abnormal heart recovery. Another study[22] demonstrated association of weight loss with significant improvement in HRR1 with no changes in peak heart rate.

Our study participants who had lower body fat ratio and BMI at baseline showed greater improvement in HRR2 after 14 days of fasting in comparison to participants with higher BMI and body fat ratio. Though individuals with both higher and lower BMI and body fat ratio at baseline showed significant improvement in HRR2, the improvement was more marked in the group with already better BMI and body fat ratio at baseline. Our findings are in agreement with the results of a previous study that showed a negative correlation of significant weight loss with heart rate recovery in gastric bypass patients.[23]

In the present study, participants with a sedentary or moderately active lifestyle showed significant improvement in HRR2 but participants who reported regular physical activity did not show significant improvement in HRR2. The changes in HRR2 after Ramadan fasting have not been evaluated, but one previous study has shown significant improvement in HRR2 in cardiac rehabilitation patients after a structured exercise program.[9] A similar study correlated improved HRR with an exercise training program in patients after anterior myocardial infarction.[24] Possible reasons for less improvement in HRR2 in our participants with an active lifestyle could be the fact that these participants already had cardiovascular adaptations as a result of metabolic and molecular remodelling associated with exercise.[25]

None of the smokers showed a significant increase of >20 bpm in HRR2 after the 14-day fasting which shows that even if fasting results in weight loss or a decrease in body fat, this improvement in body composition is less likely to be accompanied by an improvement in HRR2. Participants of our study with a body fat ratio of more than 25 at baseline were less likely to experience improvement in HRR2.

We acknowledge our study limitations in terms of a small cohort of participants that could not be reflective of the entire population and inclusion of apparently healthy males and exclusion of females. Since the main aim of the study was to explore the effects of fasting on HRR and to identify different parameters associated with abnormal heart rate recovery, it was not possible to recruit patients with cardiovascular disease, metabolic syndrome, hormonal imbalances, pregnant and breastfeeding women to avoid hazardous effects of fasting in such population.

CONCLUSION

Significant improvement in HRR2, body fat ratio, BMI and waist circumference was observed in healthy adult males after 14 days of Ramadan fasting. Randomized controlled trials with longterm follow-up periods are needed to see the impact of intermittent fasting on overall fitness and the time of onset of any cardiovascular or metabolic disease in healthy individuals. Carefully designed studies may involve individuals with cardiovascular risk factors or diabetes mellitus to explore the effects of intermittent fasting on cardiovascular fitness.

ACKNOWLEDGEMENT

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HA: Data curation**,** Methodology, Revision
HN: Visualization, Methodology, Supervision

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ORIGINAL ARTICLE

TERATOGENIC EFFECTS OF TOPICALLY APPLIED PARAPHENYLENEDIAMINE ON THE RAT FOETUS

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Background: Paraphenylenediamine (PPD) is an organic compound used in hair dye. It causes local and systemic side-effects. Not much is known regarding its foetotoxic effects. This study aimed at determining the teratogenic effects of topically applied PPD. **Methods:** This experimental study was carried out at IBMS, Khyber Medical University, and Peshawar Medical College, Peshawar over a period of 6 months. Twenty healthy Sprague Dawely female pregnant rats were divided into 5 groups, i.e., positive control (Group A), control (Group B), and 3 experimental groups (C, D and E). For group A, 0.1 ml of distilled water was applied topically on back of each rat. For positive control group B, 0.5 ml of dye containing 3% PPD was applied, while for group C, D and E, 1, 2 and 3 mg/Kg dose of PPD respectively was applied for 30 minutes daily. Skin was washed under tap water followed by rats mating. After successful fertilization, same doses were repeated for 20 days. Animals were sacrificed and foetuses were examined for skeletal abnormalities and hepatic changes. Skin biopsy of adult rats was examined for any changes. **Result:** Topically applied PPD resulted in significant teratogenic effects on foetal liver in a dose dependent manner. However, no significant teratogenic effect was observed on foetal skeleton (p=0.075). Topical PPD showed increased epidermal thickening and keratinization on adult rats skin. **Conclusion:** PPD has significant dermal effects on adult rats and teratogenic effects on liver of rat foetuses but no significant effects were observed on foetal skeleton.

Keywords: Paraphenylenediamine, Sprague dawley, Teratogenic, Topical application, Hair dye, Foetus

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INTRODUCTION

Paraphenylenediamine (PPD) is a chemical compound having formula $C_6H_4(NH_2)_2$.[1] PPD is an alanine derivative and is locally known as '*Kala Pathar*'.[2] The PPD is a black mineral from the banks of Nile River. The PPD is commercially available since 1990 and is still used in permanent hair dyes. It is also used in rubber chemicals, pigments, cosmetics and photographic development agent. PPD is a colourless grey or yellow crystalline solid, which oxidizes after exposure to air and turns red, brown, and finally black. In many countries of Africa, Middle East, and India PPD is mixed with Henna and traditionally applied to palm and soles. In USA more than one third of women use hair dye containing PPD. PPD is used to intensify the black colour of Henna and to reduce the time for dyeing as well.[3] N-acetylation catalysed by N-acetyltransferases (NATs) is major route of PPD metabolism. PPD is acetylated to monoacetyl-PPD (MAPPD), which is acetylated to Diacetyl-PPD (DAPPD).[4] PPD and these metabolites are mainly excreted through renal clearance.[5] Chronic exposure to PPD occurs in industrial workers manufacturing dyes, or in hairdressers using hair dyes containing PPD, as an occupational hazard.[1] Hair dye has been used to colour hair over the course of history. Hair dye can be categorized in terms of effectiveness and ingredients.[6] In terms of effectiveness hair dye can be temporary, semi-permanent and permanent. In terms of ingredients hair dye can be natural, mineral and artificial organic dye. Worldwide most commonly used hair dye is permanent hair dye. Paraphenylene diamine is used as an essential component of almost all hair dye formulations. The absorption of hair dye through skin varies from one person to other and depends upon person's health, gender, age and the moisture content of the dye.

For more than 4,000 years henna has been used as a cosmetic by Middle Eastern, Mediterranean and Asian cultures. Henna contains a burgundy dye molecule Lawsons (2-hydroxy-1,4-napthoquinone) which binds with proteins and is used in body art to dye hair, skin, nail and also silk, wool and leather.[7] The colour of natural henna is Orange/Reddish. PPD is mixed with natural henna to give it an ebony colour (Black Henna) and to reduce the time taken by natural henna to stain.[7]

PPD can cause local and systemic side-effects when applied topically or ingested orally. It is highly toxic, especially when taken orally.[8] Oral ingestion of PPD has been associated with systemic poisoning. The PPD induced systemic toxicity occurs in two phases. Acute phase occurs early some hours after ingestion. In acute phase tongue swelling, angioedema, burning and

numbness of the mouth, vomiting and airway obstruction occurs. The second phase occurs after a few days when toxic metabolites are absorbed and distributed throughout the body. Dark cola colour urine, acute injury to kidney, oliguria, anuria rhabdomyolysis, intravascular haemolysis and drowsiness can occur.[9] Oral ingestion of PPD has also been used for inducing abortions. A case study of a pregnant female who ingested unknown dose of PPD for the said purpose reported myocardial lysis in the aborted foetus.[10]

The lethal dose of PPD in humans varies from 7–10 gms, resulting in death due to cardiotoxicity and angioneurotic oedema.[8] In rats, a lethal dose of PDD ranges from 80–100 mg/Kg.[11] On topical application, PPD is metabolized in skin and its metabolites exert systemic effects. Hydrogen peroxide and superoxide radicles are produced as metabolites on long term exposure to PPD.[12] Interestingly, animal studies have suggested that orally ingested PPD fails to produce any teratogenic or foetotoxic effects.[13] However, one study which was carried out to determine the histopathological effects of PPD containing hair dye administered subcutaneously resulted in neonatal corneal changes.[14]

A number of studies showing the toxic effects of topically applied PPD on different adult organs have been reported.[14–19] Hair colouring products containing PPD account for 35% of non-Hodgkin's lymphoma in exposed women and 20% in all women.[5] Topically applied PPD induces haemolytic anaemia and rhabdomyolysis, which causes acute renal failure in rats.[15] The PPD also causes hepatotoxicity evident by an increase in serum biomarkers and on histopathology[16] and reduces the number of primary ovarian follicles in rat ovaries.[17] Sub-chronic topical application of PPD in different doses decreases the total sperm count and testicular weight.[18] Topically applied PPD causes histopathological changes in skin like proliferation of epithelial cells and increase keratinization.[19] Chronic dermal exposure to PPD resulted in toxic effects on pancreas that were evident from histopathological changes like vacuolation in cells of islets of Langerhans, irregular distorted acini, and congested blood vessels.[20] There is increased risk of lymphoma, leukaemia and bladder cancer in people using hair dye containing PPD.[21]

Most drugs and chemicals like tetracyclines, and anticoagulants cross the placenta. Many factors like molecular weight, fat solubility, polarity etc. determine the ability of a drug or chemical to cross the placenta and reach the foetus. High molecular weight compounds do not cross the placenta. Compounds having molecular weight 1,000 Daltons or more do not cross the placenta, while those compounds having molecular weight less than 600 Daltons cross the placenta.[22] PPD has molecular weight 108.14 Dalton so it can cross the placenta and reach the foetus and can cause teratogenic effects.

The effects of topically applied PPD have been studied in adults. So far, in literature not much evidence was retrieved regarding the teratogenic effects of topically applied PPD. No evidence was found whether topically applied PPD is capable of crossing placenta and inducing teratogenic effects or not. Since hair dyes containing PPD are frequently used among women, the rationale of this study was to understand the teratogenic effects of PPD on foetal liver, skeleton and maternal skin. This would lead to better guidance for the use of hair dyes containing PPD in pregnant women.

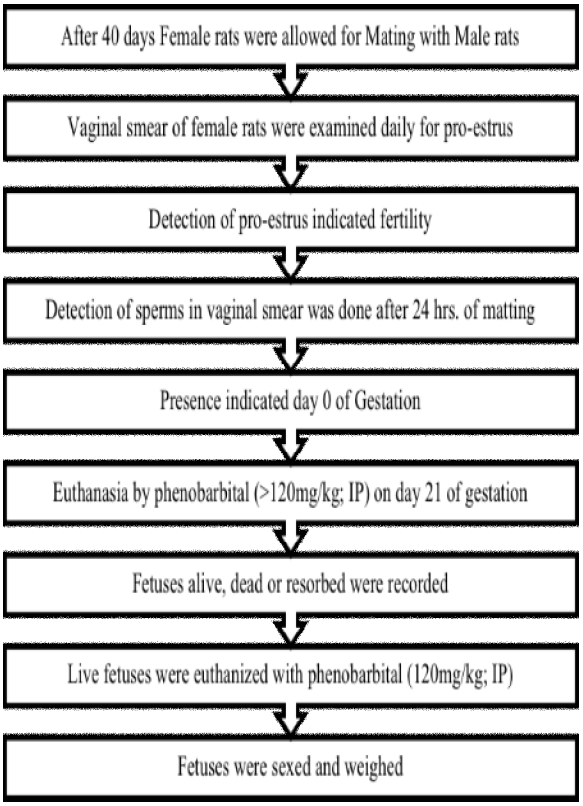
MATERIAL AND METHODS

After review and approval by Graduate Study Committee (GSC), this research proposal was approved by the Advanced Study and Research Board (ASRB) under: DIR/KMU-AS&RB/TE/001089, and Ethical Board under: DIR/KMU-EB/TE/000761. The study was carried out in the Institute of Basic Medical Sciences (IBMS) of Khyber Medical University (KMU) Peshawar, Pakistan and Peshawar Medical College Peshawar, Pakistan. This was an experimental lab-based study.

Twenty healthy Sprague Dawley female pregnant rats were taken. There were divided into five groups of four rats each as positive control, control, and three experimental groups. Sampling technique used was simple random sampling. The duration of this study was six months (June–December 2019).

The exposed surface on the dorsum of all animals were clipped free of hair two days before the topical application of PPD. PPD doses of 1, 2 and 3 mg/Kg were used for three Experimental groups C, D and E respectively. These doses were made in 1ml of distilled water and 0.1 ml of this solution was used for each dose.[15] For Group A (Control group), 0.1 ml of distal water was applied topically on exposed area of 5×6 mm^2 on the dorsal surface of each rat.[17] For Positive control group B, dye containing 3% PPD mixed with 1:1 H_2O_2, in a dose of 0.5 ml twice was applied topically.

For Group C, D and E (Experimental groups) fresh solution of 1, 2 and 3 mg/Kg dose of PPD was prepared every day in distilled water. It was applied on the exposed dorsum of each rat using plastic syringe and spreaded using spatula for 30 minutes. The skin was washed under tap water.[17] This process was repeated initially for 40 days and was then continued for 20 days after matting of rats. Total dose applied to rats over 60 days in group B was 49.5 mg, group C was 1.65 mg, group D was 3.3 mg and group E was 4.95 mg. The protocol followed to ensure mating is depicted in protocol flowchart.



Protocol flowchart

Two third of foetuses from each group were selected randomly for the examination of skeletal malformations and 1/3rd for Histopathological examination of liver.[23] Liver morphology was staged and graded on basis of Batts-Ludwig's system.[24] For isolation and examination of foetal skeleton, 2/3rd of the foetuses were fixe din 95% ethanol and defatted in 1% KOH to make tissue and skeleton visible. The skeleton was then stained with 0/0025% Alizarin red stain and 1% KOH for 24 hours. They were rinsed with water and transferred to 30% glycerine for 24 hours. Skeleton examination was carried out under dissecting microscope and foetal skeleton was observed for the skeletal abnormalities like Hemimelia, vertebral agenesis, Missing ribs, supernumerary ribs, digital defects and tail defects. For the evaluation of foetal liver, 1/3rd of foetuses were taken and their hepatic tissue was processed for Haematoxylin and Eosin staining and visualized under the light microscope.

For studying further effects of topically applied PPD we also studied on the skin sections of adult rats where PPD was applied. For skin biopsy of adult female rat, 6 mm punch biopsy was taken from the site of PPD administration and 10 micrometre cryo-sections were cut. Histological sections were fixed in 5% acetic acid in menthol. Haematoxylin and eosin staining was done to define the skin layers and structures associated with each sample for analysis of histological changes.

All the data obtained after animal experimentation was recorded in Microsoft Excel (2013). Histologic slides were analysed using image J software version 1.8.0 for determining diameter of central vein of liver and thickness of epidermis of skin. The data thus obtained was used to categorize dilation or congestion of central vein and increase or decrease in thickness of epidermis of skin. The SPSS-25 was used for all statistical analysis.

RESULTS

Number of foetuses in different groups, their male to female ratio and mean weight of foetus is shown in Table-1. The frequency of hepatic histological changes that is Periportal inflammation, Periportal necrosis, Centrilobular necrosis, Central vein dilatation and Hepatocyte hypertrophy are shown in Table-2, 3. The effects of different doses of PPD application on the skeleton of rat foetuses in different groups is shown in Table-4. In case of Periportal inflammation and necrosis the $p<0.000$ suggesting significant relationship between various PPD doses and periportal inflammation and necrosis. In case of Centrilobular necrosis, dilatation of central vein and Hepatocyte hypertrophy the p values were found to be $p= 0.03$, $p= 0.006$ and $p= 0.001$, respectively showing significant relationship between these histological changes and PPD application.

Table-1: Number of foetuses and their mean weights in each group

Groups	Number of foetuses	Male	Female	Weight in grams Mean±SD
A	21	11	10	3.39±0.18
B	24	12	12	3.68±0.30
C	33	22	11	3.78±0.63
D	24	10	14	3.5±0.18
E	30	20	10	3.51±0.30

Table-2: Frequency of periportal inflammation, periportal necrosis among foetal livers

Groups	Periportal inflammation						Periportal necrosis					
	None	Minimal	Mild	Moderate	Severe	Total	None	minimal	mild	moderate	Severe	Total
Water	7	0	0	0	0	7	7	0	0	0	0	7
Dye	0	0	0	0	8	8	2	4	1	1	0	8
1 mg/Kg	0	1	0	2	8	11	1	4	0	5	1	11
2 mg/Kg	0	0	0	1	7	8	0	1	2	2	3	8
3 mg/Kg	0	0	0	1	9	10	0	1	2	1	6	10
Total	7	1	0	4	32	44	10	10	5	9	10	44

Table-3: Frequencies of centrilobular necrosis, central vein dilatation and hepatocyte hypertrophy among foetal livers

Groups	Centrilobular Necrosis			Dilatation of central vein				Hepatocyte hypertrophy		
	Present	Absent	Total	Normal	Dilated	Congested	Total	Absent	Present	Total
Water	0	7	7	7	0	0	7	7	0	7
Dye	0	8	8	0	5	3	8	3	5	8
1 mg/Kg	6	5	11	4	6	1	11	3	8	11
2 mg/Kg	5	3	8	2	6	0	8	2	6	8
3 mg/Kg	7	3	10	4	4	2	10	0	10	10
Total	18	26	44	17	21	6	44	15	29	44

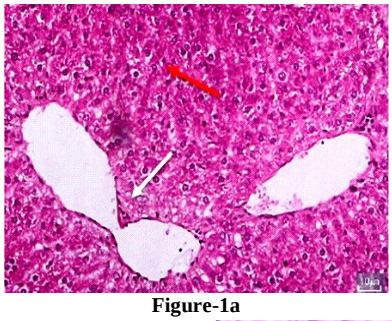


Figure-1a

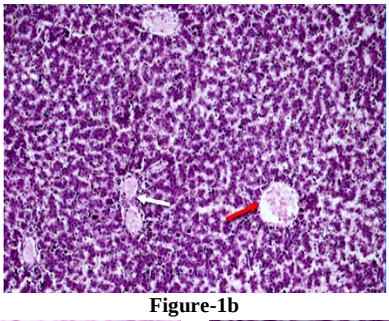


Figure-1b

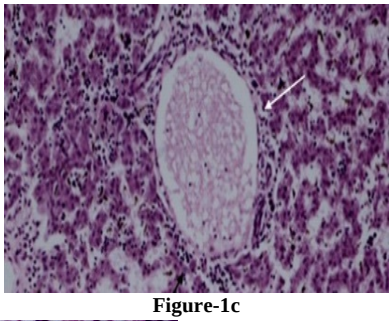


Figure-1c

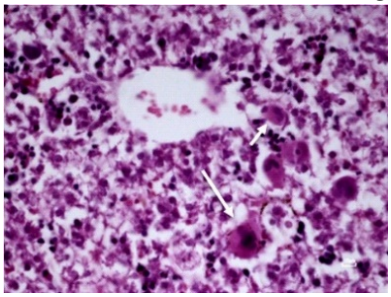


Figure-1d

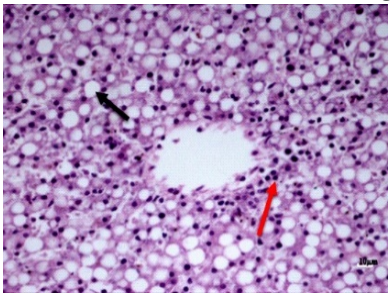


Figure-1e

Figure-1: Histology of rat foetal liver

a) normal liver histology of Control group A; white arrow is pointing toward the portal triad, red arrow points towards normal hepatic cords. b) positive control group B; white arrow showing periportal inflammation, and red pointing towards dilated central vein. c) experimental group C; white arrow showing dilated central vein, black arrows pointing hepatocyte hypertrophy with severe periportal inflammation. d) experimental group D; white arrows pointing necrotic hepatocytes. e) experimental group E; black arrow showing steatotic hepatocyte hypertrophy and red arrow pointing hepatocytes surround by infiltrative cells. A, B at 10× and C, D, E at 40×. Scale bar=10 µ

Figure-2a

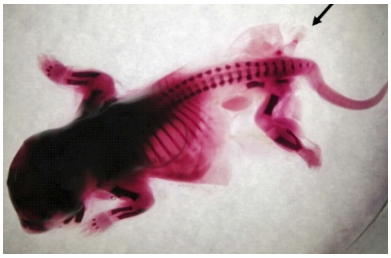


Figure-2b

Figure-2c

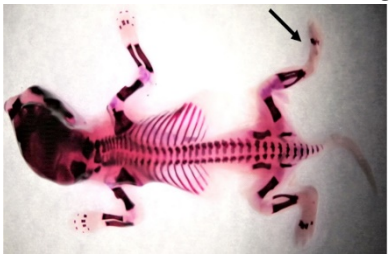


Figure-2d

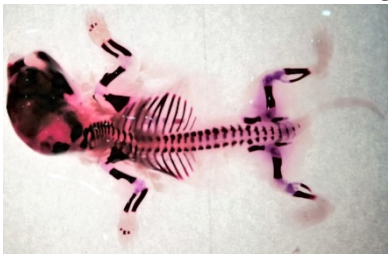


Figure-2e

Figure-2: Comparison of foetal skeleton of different groups

a) Normal foetal skeleton, b) Hemimelia (short limb) in foetal skeleton, c) Missing rib, d) Digit defect, e) Normal foetal skeleton of experimental group C

Table-4: Frequency of skeletal defects observed in each foetal group

Parameters	Animal Groups				
	A	B	C	D	E
No. of foetuses examined	14	16	22	16	20
Foetus with defects	0	0	5	2	5
Total no of defects	0	0	8	2	5
Hemimelia	0	0	0	0	1
Vertebrae agenesis	0	0	0	0	0
Ribs missing	0	0	3	0	4
Ribs supernumerary	0	0	0	0	0
Digit defects	0	0	5	2	0
Tail defects	0	0	0	0	0
Percent of foetus with defects (%)	0	0	31.25	12.5	25

As per the doses of PPD applied and frequency of defects observed, the *p* value was found to be 0.075 which was not statistically significant.

On histological examination of skin of adult rats, it was observed that topical application of different dose of PPD resulted in changes in skin usually resulting in increased keratinization and increased proliferation of epidermis resulting in increase in thickness of epidermis in all the groups except the control group.

There was a statistically significant relation between the dose of PPD, extent of keratinisation, and epidermal proliferation ($p<0.000$).

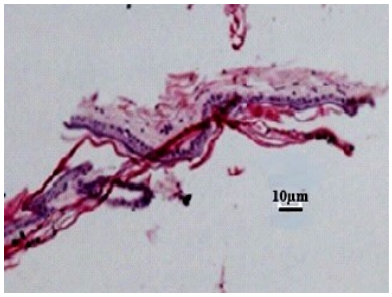
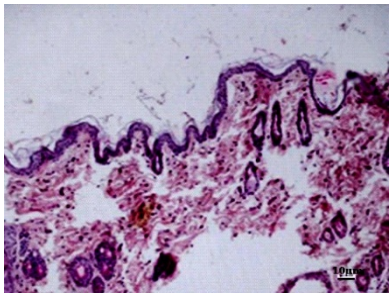


Figure-3a



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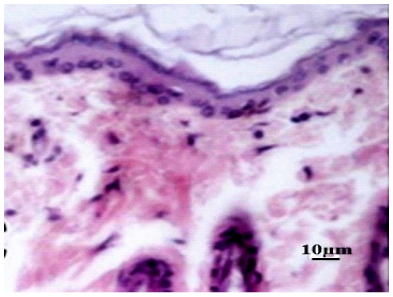

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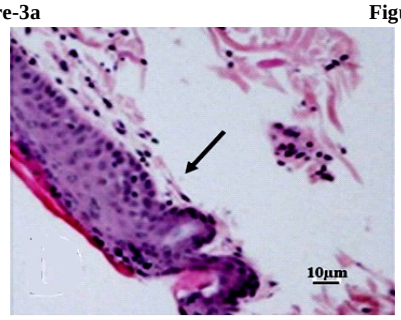

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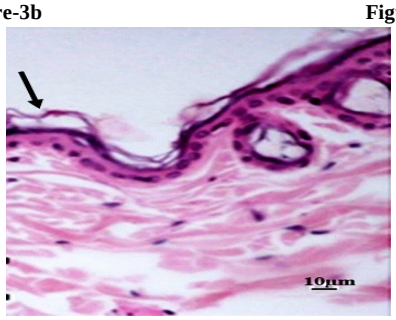

Figure-3e

Figure-3: Histologic sections of skin of adult rats of different groups

Normal skin at 10× magnification b) dye applied skin at 10× magnification c) PPD 1 mg/Kg applied skin at 10× magnification d) PPD 2 mg/Kg applied skin at 10× magnification e) PPD 3 mg/Kg applied skin at 40× magnification. Scale bar= 10 µm

DISCUSSION

This was an experimental lab-based study in which the effects of chronic application of topically applied paraphenylenediamine during gestation on foetal liver and skeleton was studied in a dose dependent manner. In addition, we looked at the local effects of topically applied PPD on the skin of adult rat. The PPD application during gestation had teratogenic effects on foetal liver. Interestingly some foetal skeletal defects were observed, however they were statistically insignificant. It was also established that chronic application of PPD leads to damaging effects on the skin of adult rat.

In this study it was found that PPD application resulted in increased incidence of all parameters of hepatotoxicity among all the study groups except for control group. Severity of hepatic injury was directly related to dose of PPD applied. These results were similar to the results found in studies of Abd-ElZaher *et al* and Bharali *et al*, where PPD induced significant increase in liver enzymes with chronic inflammation in dose dependent manner.[3,15] They studied the effects of topically applied PPD for 30 days, whereas in our study we not only examined the effects of topically applied PPD on adult rat for 60 days but also the hazardous effects of PPD on foetal liver and skeleton. We applied PPD for 40 days before matting and then 20 days after matting of the rats. Interestingly the dose of PPD applied is similar in both studies, i.e., 1, 2 and 3 mg/Kg body weight but the duration differed. They observed the changes in the adult liver parenchyma. Similar changes were observed in the foetal liver in our study. Similar inflammatory effects were found in adult rat liver after the administration of different gold nanoparticle sizes doses intraperitoneally.[25] Following Citalopram

administration in pregnant rats, inflammation of foetal liver had been observed.[26] As citalopram crosses the placenta and affects the foetus, we assume that PPD may follow the same placental transmission mechanism and cause foetal liver damage. In our study it was found that PPD had no significant teratogenic effect on foetal skeleton. Out of 88 total foetuses, skeletal defects were observed in 12 (13.6%) foetuses. Most commonly observed defects were missing ribs and digit defects followed by hemimelia. Despite occurrence of skeletal defects, no statistically significant relationship could be established with PPD exposure. This may be attributed to the limited samples size, duration of the study or the doses used in our study. How PPD have effected foetal skeletal growth needs to be studied further.

In all rats treated with PPD, epidermal thickening along chronic topical treatment of PPD in adult Sprague Dawely rats led to increased keratinization. The histopathological changes observed in our study had significant dose dependent relationship with PPD exposure. Similar results were observed by Lee *et al*[19]. Due to the production of toxic PPD metabolites in rat skin they observed decreased activity of oxygen free radical system and damaged tissue of skin suggesting that the topically applied PPD may lead to skin injury in a dose dependent manner due to oxygen free radical generation.[19] In our study also, histopathological skin changes like epidermal thickening were found to be dose dependent.

PPD is usually blended with an oxidizer to develop black colour. The allergenic hapten is produced by oxidation of PPD in epidermis or dermis. Varying degrees of skin reactions can occur due to PPD ranging from mild dermatitis, urticaria to blistering and facial edema.[27] Topically applied PPD can cause primary sensitization which results subsequently in allergic contact dermatitis. Erythema, pruritus and bullous dermatitis may manifest as skin changes.

From the above discussion it is evident that although the effects of PPD application on adult rats have been explored, the placental transmission and its fetotoxic effects need thorough consideration. Overall the study suggests that chronic topical application of PPD in doses of 1, 2 and 3 mg/Kg body weight can cause significant toxic effect on rat foetal liver and insignificant effect on rat foetal skeleton.

LIMITATIONS & RECOMMENDATIONS

We explored the histopathological features of foetal liver and gross morphological changes in foetal skeleton, the biochemical markers of hepatic injury and skeletal defects were not studied. We also did not perform chemical analysis of rat foetal blood. Further studies can be carried out to explore these aspect as it can help in measuring the dose of PPD that crossed placenta and can relate teratogenic effects with actual absorbed dose. The current study showed that application of the PPD on the skin of adult rats not only resulted in local damage to the skin but also had teratogenic effects on the liver and skeleton of the rat foetuses. Therefore, replacing hair dyes containing PPD with less harmful ones will be an urgent challenge not only from the prospective of chemical formulation but also from risk assessment prospective as well. It is necessary to take safety measures during the handling of PPD containing hair dyes for sufficient protection from its local and teratogenic effects. PPD is prohibited for use on skin because of its dangerous chemical nature. It is only allowed to be used in hair dyes when the hair dyes do not touch the scalp. Further experimental research work is required to confirm the effects of PPD mediated dermal changes in adults and its teratogenic effects on foetal liver and skeleton. Also skin at the site of application can be looked for loss of appendages such as hair follicles etc.

CONCLUSION

PPD has dose dependent statistically significant teratogenic effects on the liver of rat foetuses in terms of increased periportal inflammation and necrosis, centrilobular necrosis, dilatation of central vein and hepatocyte hypertrophy. PPD does have teratogenic effects on rat foetal skeleton in terms of missing digits, missing ribs and hemimelia but these effects were not statistically significant. PPD has statistically significant effects on the skin of adult rats in terms of histological changes, i.e., increased keratinization and proliferation of epidermis.

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NB: Concept, Results interpretation, Critical review
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ZH: Critical review and revision
IG: Statistical analysis, Tabulation
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ORIGINAL ARTICLE

EFFECTS OF METFORMIN AND INSULIN ON MORPHOLOGY, STEREOLOGY AND MEAN MORPHOMETRIC DIFFUSION CAPACITY IN DIABETIC PLACENTA

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Background: Insulin given for the treatment of GDM is associated with hypoxic changes whereas Metformin has beneficial micro-vascular effects on the placenta. This comparative drug study was conducted with objectives to compare the effects of Metformin and Insulin on the morphology and stereology in gestational diabetes. And to calculate and compare the mean morphometric diffusion capacity for oxygen in Metformin and Insulin treated gestational diabetics. **Methods:** This clinical trial was conducted from Jan 2018 to Feb 2019 in the Department of Pharmacology, Basic Medical Sciences Institute, in collaboration with the Department of Obstetrics and Gynaecology, Jinnah Postgraduate Medical Centre, Karachi. Out of 136 high-risk females, 83 confirmed gestational diabetics were enrolled in second trimester and were further randomised into Group A (oral Metformin therapy) and Group B (subcutaneous Insulin therapy). They were followed till term and after delivery. Collected placentae (35 in each group) were subjected to gross, microscopic and detailed stereological study. **Results:** Placental weight on gross, hypoxic changes such as immature villi, chorangiosis and syncytial knots on light microscopic morphology, placental volume, the total volume of placental villi and total volume of foetal connective tissue on stereology were significantly more in Insulin-treated diabetic placentae whereas the mean villi diameter and mean morphometric diffusion capacity was significantly more in Metformin-treated placentae. **Conclusion:** Metformin-treated placentae were significantly different as compared to the insulin-treated placenta. Metformin treated placentae showed better morphometric diffusion capacity for oxygen than insulin treated placentae.

Keywords: Gestational diabetes mellitus, Insulin, Metformin, Placenta, Stereology

Pak J Physiol 2022;18(2):22–7

INTRODUCTION

Stereology is the study of tissues that impart practical lengths, along with densities for obtaining numbers of important structures in microscopic sections.[1] This system works with an applicable approach, usually composed of a grid of points or lines, applied to the area required to estimate numerical evaluation and area densities of biological structures.[2,3]

Gestational diabetes is the raised sugar level in maternal blood (FBS >100 mg/dl, RBS >126 mg/dl) as gestational hormones decrease insulin effectiveness, causing multiple maternal and foetal complications.[4] Placenta performs many important functions compulsory for the growth of the foetus. High maternal glucose level causes histological alterations in the placenta such as augmented number of vessels in villous (chorangiosis), distorted development of villi, syncytial knots, areas of fibrosis and ischemia and also on stereological details, increased surface area, total volume, the volume of inter-villous space and terminal villi.[5] These modifications affect placental physiology.[6] Furthermore, these placental morphological changes are responsible for the decrease in the oxygen delivery to the foetus due to a relative increase in size and amount of villous and vascular sections, with the increase in thickness of the basal membrane and multiple cellular depositions.[7]

Thus, Gestational diabetes mellitus demands early diagnosis and suitable treatment to reduce the feto-maternal problems coupled with it.[8] Inject-able Insulin was considered to be the best but; due to anabolic effects of insulin, multiple changes in placental morphology are documented with it with unfavourable gestational outcomes. Another option is the oral drug Metformin, which provides better compliance with added valuable effects as indicated by improved gestational consequences. However, whether Metformin compared to Insulin produces less hypoxic changes in the placenta on stereology was still left to be addressed. This study was conducted to evaluate and compare the stereological details of the placenta of mothers given Metformin *vs* Insulin for GDM.

METHODOLOGY

This clinical trial was carried out from Jan 2018 to Feb 2019 at Basic Medical Sciences Institute, in collaboration with the Department of Obs/Gyn, Jinnah Postgraduate Medical Centre (JPMC) after approval from the Institutional Review Board, JPMC, Karachi and BASR, University of Karachi. The study was further registered with clinicaltrial.gov (NCT number: NCT04907708).

The Sample size was calculated using 'Comparing of Two Means' on www.Openepi.com by a reference article with a mean difference of surface size of placental morphology, at the power of 80% and confidence level of 95%, the minimum sample size came out to be 23 in each group (raised to 35).[9]

After written informed consent, induction of diagnosed GDM patients was done as per WHO criteria (FBS >100 mg/dl, RBS >126 mg/dl) and out of 136 high-risk females, 83 confirmed GDM patients were enrolled. They were further randomised to Group A, 42 GDM patients were prescribed oral Metformin therapy (500 mg, TDS with dose escalation therapy) along with adjuvant diet control Whilst 41 patients in Group B were prescribed subcutaneous Insulin (0.8–0.9 IU/kg/day) with a strict nutritional check. They were monitored till the term and, after delivery, a comprehensive stereological study was done on saved placentae.[10]

Data were evaluated for 35 placentae in each group who were able to complete the study. After gross examination, histological slides were made for light microscopy. These slides were analysed on a light microscope for villous immaturity, chorangiosis, infarction, ischemia, villous fibroid necrosis, nucleated foetal RBCs, calcification and syncytial knots.

For stereology, placentae were assessed for volumes of components using a Nikon microscope (Eclipse 50i) attached to the DS-camera control unit DSL2 (M380E). Ten randomly selected microscopic fields were taken from two selected slides either from 12 O'clock, 6 O'clock and Centre positions from each placenta. The microscope stage was blindly and randomly displaced for the stereological assessment using a point-counting method to reveal the volume of placental components.[11]

From the slide, five sections were studied in detail (a total of 350 sections in each group). A 100 squared grid with 1 Cm of each square, was overlaid on the microscopic fields and for density of placental structures, intersecting points of the grid lying on these placental structures were counted. Volume density of each component (villi, inter-villous space, foetal capillaries and foetal connective tissue) was obtained by: Volume Density of placental component (Vd)= Number of intersecting points of grid observed on the object of focus/Total number of points used in the relevant grid section[11]. (Figure-1, 2).

The placental volume was estimated (V=W/D)[10] with the density of 1.05−3 gm and total volume for all placental components (villi, inter-villous space, foetal capillaries and foetal connective tissue) was calculated. (Vd×V)

The diameter of the villi and the foetal capillary was measured through the measuring scale available inside the computer and microscope. The Surface area of placental villi (SA villi) and capillaries (SA cap) was calculated by the intersection counting method for which a horizontal line grid was used.

SA villi=2×No. of Intersections of horizontal lines made by villi (I_V)/Lt)
SA cap=2×No. of Intersections of horizontal lines made by foetal capillaries (Ic/Lt)

where 'I' is the mean of villi and capillary intersections with horizontal lines whereas 'Lt' is the total length of grid horizontal lines.[11] (Figure-3).

Orthogonal intercepts are the horizontal or vertical lines perpendicular to the villous membranes, were also determined using a similar square grid. Arithmetic mean and the harmonic mean was calculated and for random plane sectioning, the harmonic mean was finally multiplied with 0.848.[12] (Figure-4).

The mean of diffusion capacity for oxygen across the villous was calculated as:

Mean diffusion capacity (villi)=MDC (villi)=Surface area of exchange (S.A v+S.A cap)×Krogh's constant for O_2 (2.3×10^{-8})/2×Harmonic mean thickness (VM)

This represents almost 90% of the diffusion capacity of oxygen across the placental membranes.[13]

Collected data were analysed and statistical tests were applied accordingly for categorical and numerical values, and $p<0.05$ was considered significant.

RESULTS

The patients enrolled were similar except for random blood sugar levels ($p=0.00$) (Table-1).

The gross examination revealed that the size, length, width and breadth of the placenta across the two groups was non-significant and the only mean weight of the placenta was statistically different between the groups. ($p=0.00$) (Table-2).

On light microscopic examination, significantly more villi were immature, more chorangiosis and Syncytial knots in group B placentae were observed. (Table-3)

On stereological comparison, group B placentae were significantly more in total volume ($p=0.02$), the total volume of foetal connective tissue ($p=0.04$) and the mean volume of villi ($p=0.05$). The mean diameter of villi was more in Group A placentae ($p=0.05$) and the MMDC was significantly different between the groups with better values in Group A ($p=0.04$) (Table-4).

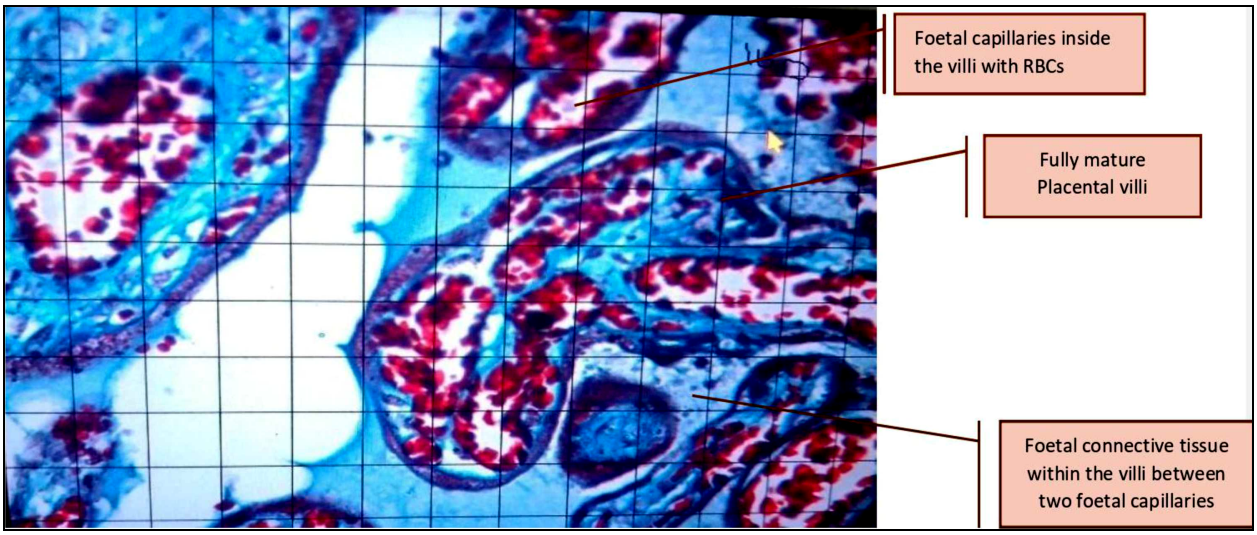

Figure-1: Grid imposed on a histological section of Metformin treated placenta. Intersecting points are counted on different components of placenta. ×40

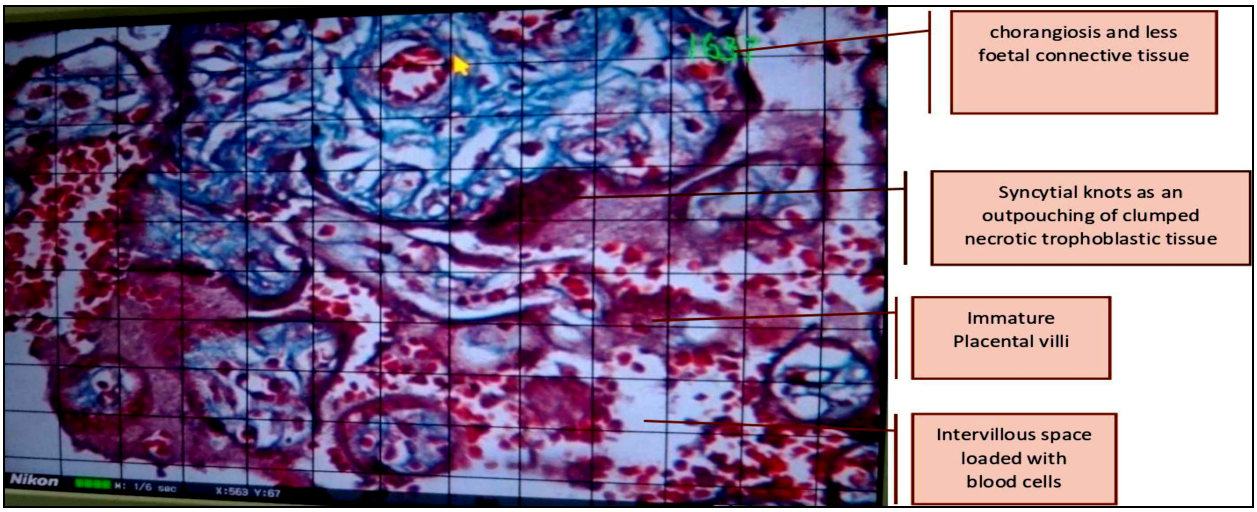

Figure-2: Grid imposed on a histological section of insulin treated placenta. Intersecting points are counted on different components of placenta. ×40

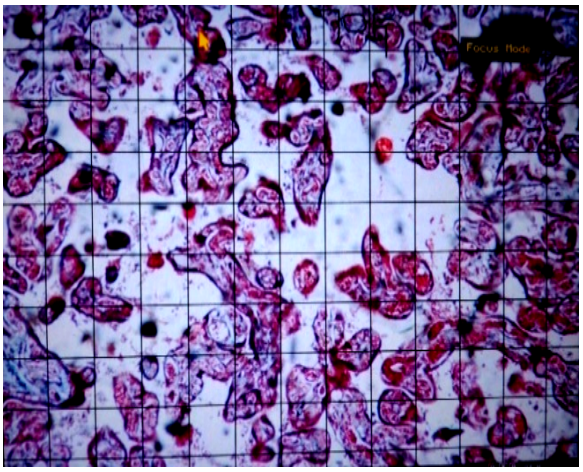

Figure-3: The section of placenta for intersection counting in horizontal lines ×10

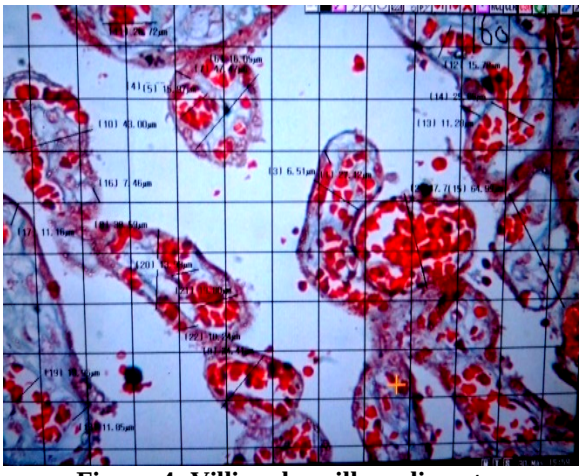

Figure-4: Villi and capillary diameter measurement

Table-1: Maternal characteristics (Mean±SD)

Characteristics	Group A n=35	Group B n=35	*p*
Age (years)	31.05±3.97	31.74±4.00	0.47
Weight (kg)	81.53±10.53	77.5 ±8.43	0.07
Height of the fundus (weeks)	31.31±2.77	30.97±2.85	0.61
FBS-1 (mg/dl) (>100mg/dl)	107.65±13.57	115.25±25.42	0.12
RBS (mg/dl) (>126mg/dl)	163.34±35.29	223.62±68.94	0.00*
HBAIC_1 (5.5–6.5) (%)	5.32±0.43	5.44±0.31	0.17

*Statistically significant (independent *t*-test applied); FBS: fasting blood sugar, RBS: random blood sugar; HBAIC 1: Glycated sugar at enrolment

Table-2: Placental gross morphological features (Mean±SD)

Characteristics	Group A n=35 Mean±SD	Group B n=35 Mean±SD	*p*
Placental surface size 1cm	15.25±2.58	16.22±3.2	0.16
Placental surface size 2cm	13.71±2.35	13.74±2.3	0.95
Placental width (cm)	2.3±0.59	2.37±0.68	0.64
Placental weight (gm)	626.85±115.0	712.28±110.56	0.00*

*Statistically significant (independent *t*-test applied)

Table-3: Microscopic morphology of placenta [n (%)]

Microscopic Examination	Group A (n=35)	Group B (n=35)	*p*
Villous immaturity			
Present	13 (37.1)	24 (68.5)	0.00*
Not present	22 (62.8)	11 (31.4)	
Chorangiosis			
Present	8 (22.8)	22 (62.8)	0.00*
Not present	27 (77.1)	13 (37.1)	
Infarction			
Present	10 (28.5)	10 (28.5)	>0.9
Not present	25 (71.4)	25 (71.4)	
Villous fibrinoid necrosis			
Present	23 (65.7)	26 (74.2)	0.43
Not present	12 (34.2)	9 (25.7)	
Nucleated RBCs			
Present	0 (0)	1 (2.8)	NA
Not present	35 (100)	34 (97.1)	
Ischemia			
Present	6 (17.14)	5 (14.2)	0.74
Not present	29 (82.8)	30 (85.7)	
Calcification			
Present	15 (42.8%)	8 (22.8%)	0.07
Not present	20 (57.1%)	27 (77.1%)	
Syncytial knots			
Present	10 (28.5)	20 (57.1)	0.01*
Not present	25 (71.4)	15 (42.8)	

*statistically significant (chi-square applied),
NA: Chi-square not applicable due to less cell count

Table-4: Stereological measurements group A *vs* B

Parameters	Group A n=35	Group B n=35	*p*
Villi volume density (gm/Cm^3)	55.59±11.15	55.79±9.75	0.93
Inter-villous space volume density (gm/Cm^3)	48.73±10.66	46.59±11.04	0.41
Foetal capillaries volume density (gm/Cm^3)	15.12±5.7	18.56±22.14	0.37
Foetal connective tissue volume density (gm/ Cm^3)	38.92±7.74	37.00±9.21	0.34
Placental volume (Cm^3)	597.51±108.14	659.72±118.52	0.02*
Total volumes of placental villi (Cm^3)	312.23±88.35	349.09±65.85	0.05*
The total volume of the Inter-villous area (Cm^3)	274.54±84.77	283.52±76.26	0.64
The total volume of foetal capillaries (Cm^3)	82.52±45.25	94.40±22.91	0.17
The total volume of foetal connective tissue (Cm^3)	229.48±62.04	256.79±50.44	0.04*
Mean villi diameter (Cm)	55.05±10.51	49.44±12.88	0.05*
Mean foetal capillary diameter(Cm)	18.49±6.98	20.49±7.58	0.25
Villous surface density (gm/ Cm^3)	9.11±3.38	9.20±2.79	0.90
Capillary Surface density (gm/ Cm^3)	8.32±2.71	8.44±2.44	0.85
Harmonic mean thickness of Villous membrane (Cm)	8.03±2.67	8.42±2.02	0.55
Corrected harmonic mean diameter (Cm)	6.7±2.17	6.98±2.25	0.59
Morphometric diffusing capacity (Cm^2.min-1. kpa1)	3.66±1.43	3.02±1.15	0.04*

Group A: Metformin with diet control group= diabetic pregnancies
Group B: Insulin with diet control group = diabetic pregnancies
*statistically significant (students *t*-test applied)

DISCUSSION

Stereology is an elaborated study to provide the quantitative assessment of densities and volumes of different important elements such as villi, inter-villous space, foetal capillaries and foetal stroma.[14] Various volumes of placental elements indicate a thorough interpretation for the exchange of gases between the foetus and placenta.[15] The malformation of these components, increase in the thickness of the endothelial cells and thickening of basal membranes of the trophoblast can lower the oxygen diffusion across the placental bed.[16]

Hypoxia leads to development of many free radicals and super-oxides that are responsible for placental cell damage and apoptosis. Later on this leads to serious pregnancy outcomes.[17]

When we compared the two group placentae grossly, it was revealed that the placentae were significantly heavier in the Insulin-treatment group. Different studies have documented that the placenta of patients treated with insulin was significantly heavier compared to normal placenta.[18] Our study results reveal that insulin-treated placentae showed morphology with more hypoxic changes. Another study declared that the villi in the Insulin-treated placenta were significantly showing hypoxic morphological changes with immature villi and syncytial knots formation. Even with more weight and larger sizes of the placenta, the foetal maternal outcomes in the Insulin-treated patients were not satisfied thus confirming the fact that immature villi and more degenerative morphology can lead to adverse outcomes in Insulin-treated patients.[19] It is indicated that the placentae of Insulin-treated mothers had significantly higher volumes, due to the anabolic action of Insulin along with multiple growth mediators released to compensate for the hypoxic environment in these placentae. This fact has already been documented

that the terminal villous volume was significantly more in GDM, compared to the control group.[7] In another study, it was observed that the diabetic placenta has significantly more inter-villous and blood vascular volume when compared to the control group.[20] However, none of these studies has provided stereological comparative differences in details of placenta with metformin *vs* insulin treatment. A study[21] on Insulin-dependent patients showed placental stereology resulting in significantly increased placental weight, placental volume and villi volume and is similar to our results. An increased volume of villi and foetal connective tissue can be related to compensating for the chronic foetal hypoxia. Recently, a suggested hypothetical model for the insulin-treated diabetic placenta showed that intrauterine hypoxia increases surface area for oxygen exchange by increasing the volume of villi. But these villi lack proper maturity and are unable to compensate for the increased oxygen demand of macrosomic babies leading, at times, to unexplained intrauterine deaths.[22]

Our study results indicated that the placentae in cases treated with Metformin had significantly more dilated, fully mature maternal villi. This shows better maternal blood flow due to Metformin's favourable vasculo-protective effects which helps in free and maximum delivery of oxygen to the growing foetus. The less volume of inter-villous space in these placentae also favours the more rapid entry and delivery of oxygen to the baby. This was further proven when MMDC was calculated and was significantly more when compared with placentae of insulin-treated cases. MMDC is a diffusive conductance and can be calculated by evaluating stereological estimates of placental structural quantities. This approach has proved valuable in a variety of comparative studies.[23]

There was a significant increase in placental weight, placental volume, volume of the inter-villous space and the trophoblasts in the diabetic group with a significant reduction in the villous membrane specific diffusing capacity between the diabetic *vs* control groups, though we were unable to find any stereological studies for the metformin-treated placentae alone or with insulin. Jauniaux study results were just similar to our results for insulin-treated diabetics for GDM, indicating that reduction in the diffusing capacity of the villous membrane surely contribute to the foetal hypoxia and increased foetal and neonatal morbidity in insulin-treated diabetics.[20]

A recent meta-analysis on 4,533 women from 23 clinical trials has also confirmed that metformin has shown the most promising results in successfully controlling glycaemia and preventing neonatal complications in GDM patients as compared to other pharmacological interventions, and one of the probable reasons along with good glycaemic control could be its vasculo-protective beneficial effects on the placental tissues.[24]

CONCLUSION

Metformin-treated placentae had mature villi with significantly more mean villi diameter and mean morphometric diffusion capacity for oxygen, making it a better alternative for gestational diabetes.

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RA: Conceptualization, and designed the study, collected data, and did main write up of manuscript.
FA: Analysed and interpreted data, drafted manuscript reviewed proofread final manuscript.
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ORIGINAL ARTICLE

CORRELATION OF SERUM GALECTIN-3 LEVELS WITH EJECTION FRACTIONS IN HEALTHY SUBJECTS AND HEART FAILURE PATIENTS

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Background: High Galectin-3 levels are indicative of severity of heart diseases and are associated with increased risk of major adverse cardiovascular events including heart failure. The objective of this study was to determine correlation between ejection fraction and serum Galectin-3 levels recorded by simple and quick test. **Methods:** This cross-sectional analytical study was conducted at the Armed Forces Institute of Cardiology (AFIC) Rawalpindi. Thirty (30) healthy adults and 60 heart failure patients were selected through randomised sampling. Diagnosed cases were classified using modified NYHA classification. Group 1 included healthy adults, Group 2 included 30 mild heart failure subjects of NYHA class I and II, Group 3 included 30 severe heart failure subjects of NYHA class III and IV. Ejection fraction was recorded at AFIC with trans-thoracic echocardiography in supine and left lateral positions. Blood samples of the participants were used for estimation of Galectin-3 by ELISA using human serum Galectin-3 ELISA kit Catalogue No. E1951Hu (Bio Assay technology). Observations were recorded on proforma, Pearson's correlation test and ANOVA were performed using SPSS-24. **Results:** The LVEF was 70.7±2.78% in Group-1, 59.3±3.4% in Group-2, and 38.96±12.27% in Group-3. The LVEF showed a substantial negative correlation with serum Galectin-3 levels (r= -0.630, p<0.001). **Conclusion:** There is a significant negative correlation between Serum Galectin-3 and extent of functional changes of left ventricle recorded by Ejection fraction on echocardiography.

Keywords: Heart Failure, Galectin-3, Echocardiographic parameters, Ejection fraction

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INTRODUCTION

Heart failure (HF) is a terminal cardiac disease and carries risk of morbidity and mortality. It has multiple aetiologies and many of the cardiovascular diseases and coronary artery diseases end up in heart failure. Heart failure can be categorized by cardiac remodelling; ventricular dysfunction and fibrosis.[1] Several biomarkers have been established for diagnosis and prognosis of heart failure. One novel blood marker approved by Food and Drug Administration for its role in fibrosis is Galectin-3. Various recent clinical studies show that Galectin-3 mediates fibrosis in a variety of organ systems including heart, thus it is considered as a biomarker that reflects ventricular remodelling.[2] In Pakistani population, however, no such studies have been carried out and such correlation has not been determined.

Galectin-3 belongs to multifunctional lectin family.[3] It is a protein that serves to bind Galactoside, thus helping in cell-to-cell adhesion and cell matrix interactions. Galectin-3 causes myofibroblast proliferation, fibrogenesis and inflammation.[4] Echocardiographic parameters give us an idea of heart functioning, structure, remodelling and severity of heart disease.[5] Cardiac pathologies bringing about a change in functioning of heart result in altered ejection fraction and can be assessed by echocardiographic measurement.

In recent times of pandemic outbreak where emergency patients were not immediately put on echocardiography machine until the COVID-19 results were available, there was a need for a simple and affordable test that could provide the assessment of ventricular remodelling and functioning. Serum Galectin-3 levels could prove to be the missing indicator. This study aimed to provide a biomarker assessment in heart failure patients for determining the extent of ventricular remodelling and associated ejection fraction changes. This study can fill in the existing knowledge gap regarding South-Asian population in general and Pakistani population in particular.

METHODOLOGY

This study was conducted at Army Medical College and Armed Forces institute of Cardiology (AFIC), Rawalpindi after the formal approval from ethics review boards. The Heart failure patients admitted or visiting outpatient department (OPD) from July 2020 to October 2020 were selected. Sampling technique was Non probability purposive and WHO calculator of sample size was employed for calculating sample size. Heart failure patients were diagnosed on the basis of Framingham's criteria and classified using New York Heart Association (NYHA) classification into class I–IV depending upon severity. For our study we took ninety

adults (age range 19–65 years) and divided them into three groups using modified NYHA classification. Group 1 included healthy adults. Group 2 included 30 mild heart failure patients in NYHA class I and II whereas Group 3 included 30 severe heart failure patients in NYHA class III and IV.

Participants were assessed for the fulfilment of inclusion criteria and those having endocrine abnormalities, inflammatory disorders, cancers and using anti-inflammatory drugs were excluded from the study. All participants provided a written informed consent. Their demography and history were recorded and physical examination was carried out. Levels of Galectin-3 were recorded from blood serums of all the participants by ELISA.

The procedures followed were consistent with the ethical standards of Armed Forces Institute of Cardiology ethics review board. The research was designed in accordance with the Helsinki Declaration 1975, as revised in 1983.

We took measurements of 90 participants divided into three groups based on severity of Heart Failure using the modified NYHA classification. Ejection fraction was recorded and calculated on Trans-thoracic echocardiography. Means of Ejection fraction for each group were also recorded.

Serum Galectin-3 levels were recorded from the blood samples of all participants using Enzyme linked Immunosorbent Assay human serum Galectin-3 kit.[6] The study variables Left ventricular Ejection Fraction (LVEF) and serum Galectin-3 levels are quantitative hence these were correlated using Pearson's correlation. The data was analysed using SPSS-24. The ejection fraction and Galectin 3 were recorded in the three study groups.

RESULTS

With worsening heart failure, serum Galectin-3 levels increased indicating underlying processes of myocardial remodelling and fibrosis. The mean ejection fraction of the group 1 (healthy adults) was found to be 70.7±2.78%, for group 2 (mild heart failure NYHA I and II)) it was 59.3±3.4% and that of group 3 (severe heart failure NYHA III and IV) it was 38.96±12.27% as shown in Table-1. There is a significant negative correlation between ejection fraction and serum Galectin-3 levels (r= -0.630, p<0.001) (Figure-1).

Table-1: Ejection fraction in healthy adults, mild heart failure patients and severe heart failure patients (Mean±SD)

Echocardiographic Parameter	Group-1 Healthy adults	Group-2 NYHA (I & II)	Group-3 NYHA (III & IV)	*p*
LVEF (%)	70.7±2.78	59.3±3.4	38.96±12.27	<0.001*

*Significant

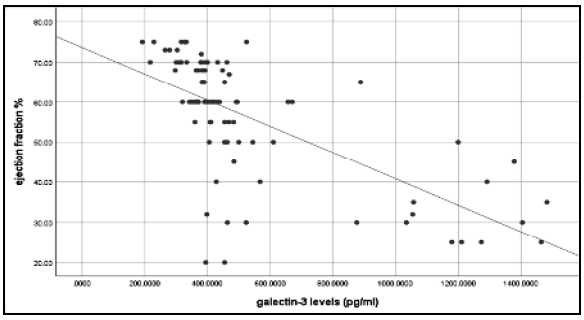

Figure-1: Correlation of serum Galectin-3 and LVEF

DISCUSSION

We associated left ventricular ejection fraction changes to levels of a biomarker which could be recorded by a quick blood test. These levels could help during the initial management and risk stratification of the heart failure patients especially in pandemic conditions where other investigations were difficult and restricted.

In this study left ventricular ejection fraction was recorded in all 90 subjects and Pearson's correlation analysis was used to find correlations between the recorded Ventricular ejection fractions and the Galectin-3 levels. With progressive decrease in ejection fraction, greater Galectin-3 levels were seen depicted by significant and negative *r* values.

Recent studies have evaluated the relationship between ejection fraction and Galectin-3 levels systematically. The Deventer-Alkmaar Heart Failure Clinic Project: DEAL-HF trial conducted echocardiographic evaluation of 240 heart failure patients and found a negative association between serum Galectin-3 and LVEF.[7] In a study on 100 patients with LV dysfunction Pearson correlation analysis was conducted to correlate the levels of serum Galectin-3 with echocardiographic indices of HF patients. The levels of serum Galectin-3 had highly significant negative correlation with LV ejection fraction.[8] In a study including 63 cases of heart failure with ejection fraction ≥50%, confirmed with echocardiography, Galectin-3 levels in serum were measured using an enzyme-linked-immunosorbent serologic assay. The values were significantly higher in heart failure cases compared with the control group.[9] Yin *et al*[10] found the mean Galectin-3 levels significantly higher in HFpEF patients versus healthy controls (23.09 *vs* 16.74 ng/mL, p<0.0001). Another study showed similarity in results when Pearson correlation analysis was conducted to correlate the levels of serum Galectin-3 with echocardiographic indices of HF patients. Galectin-3 levels had highly significant negative correlation with LVEF (p<0.001).[11] A Research conducted on 115 patients of heart failure revealed a negative correlation between the LVEF and Galectin 3 levels (r= -0.139).[12] Our findings are in agreement and coherent with these observations.

There have been contradicting studies as well. In a study on Chinese population including 133 chronic HF patients and 45 decompensated HF patients no association between myocardial function (indicated by echocardiographic reading such as LVEF) and plasma concentrations of serum Galectin-3 was found.[13] There is strong evidence of reduced efficiency of Galectin-3 as a bio-marker in various ethnicities. In a sub-study of the Atherosclerosis Risk in Communities observational cohort including 1,375 white patients and 434 black patients, Galectin-3 was found to be associated with heart failure and mortality among white patients and not among black patients.[14]

No such studies have been conducted on Pakistani population until now providing a room for further study to fill the knowledge gap. Our study was a cross-sectional study and the levels of biomarkers were measured at a single time; it is therefore possible that the longitudinal trajectory of change in biomarkers may provide additional causal inferences.

CONCLUSION

Galectin-3 levels are associated with abnormalities of myocardial function related to heart failure. Serum Galectin-3 is a diagnostic biochemical marker for heart failure and indicates severity of disease quantitatively. Serum Galectin-3 level is closely correlated to the degree of left ventricular structural and functional changes observed on echocardiography in heart failure.

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ORIGINAL ARTICLE

PRE-DIAGNOSTIC SERUM LEVELS OF INFLAMMATORY CYTOKINES IN PATIENTS WITH FIBROMYALGIA

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Background: Fibromyalgia is characterised by fatigue, sleep disruption and pain perception. The aetiology of this syndrome is still unclear. Central sensitization is considered to be a major mechanism of action and other factors may be involved including hormonal, genetic and immunological factors. The objective of the present study was to evaluate the role of different interleukins and matrix metalloproteinases in the development of fibromyalgia. **Method:** Complete blood count (CBC) profile of 50 patients and 50 controls were measured on automated haematology blood analyser. The levels of Interleukins IL-1, IL-2, IL-6, IL-7, IL-8, IL-9, IL-17, IL-18, IL-1β and IL-1-RA were measured using commercially available kits. Matrix metalloproteinases (MMP)-2, 7, and 9 were analysed by ELISA kit assay. **Results:** The mean values of IL-1, IL-2, IL-6, IL-7 and IL-9 were significantly increased in the patients with fibromyalgia when compared to control individuals. Elevated trends of IL-8, IL-17, IL-18, IL-1β and IL-1-RA were recorded in the patients with fibromyalgia as compared to control individuals respectively. The mean values of MMP-2, MMP-7 and MMP-9 were significantly increased in the patients with fibromyalgia as compared to control individuals. **Conclusion** The levels of inflammatory markers such as IL-1, IL-2, IL-6, IL-7, IL-8, IL-9, IL-17, IL-18, IL-1β and IL-1-RA were significantly higher among the fibromyalgia patients as compared to controls. These inflammatory markers may have prominent role in the development and progression of disease condition.

Keywords: Fibromyalgia, matrix metalloproteinases, MMPs, interleukins

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INTRODUCTION

Fibromyalgia belongs to the family of related disorders, which are called affective spectrum disorders. These disorders have genetic risk factors and physiological abnormalities that might be considered as central aetiology of the disease.[1] Affective spectrum disorders include a variety of psychiatric disorders such as bulima nervosa, premenstrual dysphoric disorder, major depressive disorder, panic disorder and various medical disorders (for example, cataplexy, migraine, irritable bowel syndrome). Pathophysiology of fibromyalgia includes multiple factors such as genetic disorders, autonomic nervous system disorders, environmental stressors and psychosocial variables. These factors are also linked with other disorders that commonly occur in fibromyalgia and are distinguished by emotional distress and recurrent or persistent pain.[1] Fibromyalgia may also occur with chronic inflammatory diseases including systemic lupus erythematosus, osteoarthritis and rheumatoid arthritis. The occurrence of one or more of these conditions may serve as the hallmark for the diagnosis and management of fibromyalgia. The patients suffering from fibromyalgia have increased sensitivity due to various stimuli including cold or heat as well as ischemic and mechanical pressure. In the fibromyalgia patients stimuli to the intensity of the pain varies among one to another.[2]

Fibromyalgia is distinguished as augmentation of sensory input, which is induced by central nervous system events that is linked with neuropathic pain complications such as central sensitization. Moreover, fibromyalgia patients also show abnormal levels of norepinephrine and serotonin, which are important neurotransmitters in pain inhibitory mechanism. In human and animal models of neuropathic pain, the source of nerve injury is identifiable, and pain perception might be decreased, once the source is eradicated. The study has reported the painful symptoms of fibromyalgia include abnormality in the descending pain inhibitory mechanisms. The conduction of sensory input into the brain is blocked by stimulation of fibres, which descend from the brain stem regions to the dorsal horn and resultantly cause the secretion of neurotransmitters. The patients with fibromyalgia have pain inhibitory system that can be altered in response to the deficiency of the central nervous system. These patients have low concentration of serotonin, dopamine and norepinephrine.[3] Fibromyalgia is also believed to be a stress related disorder, which includes impaired functioning in the hypothalamic pituitary adrenal axis. It is also linked with failure to reduce cortisol concentrations. It has been reported that the patients with fibromyalgia have increased concentration of cortisol as compared to control individuals.[4] In addition, these patients have disturbances in hypothalamic pituitary axis functioning, such as increased levels of cortisol and blunted stress response to ovine corticotrophin releasing hormone. Aberrations in the

activities of autonomic nervous system are recorded in these patients. These abnormalities may include various clinical problems and increased pain perception linked with fibromyalgia through modification of physiological processes, which are required for stress management and pain suppression by decreased synthesis of insulin like growth factor and growth hormone.[4]

The alternations in the autonomic nervous system activity consist of orthostatic hypotension and micro-circulatory hypotension. The patients have low microcirculatory responses to auditory activation and blunt vasoconstriction activity due to cold presser tasks as compared to control individuals.[5] In addition, genetic predisposition is one of the important factors for the progression of this disease. There are various types of genes, but the most important one is linked with neurotransmitters. The transporters of serotonin gene maybe distinguished by single nucleotide polymorphism and considered more frequent in the patients affected by fibromyalgia and psychological stress.[6] Other genes are involved are HLA region, Catechol-O-methyl-transferase gene and dopamine D4 receptor gene in the disease progression. Fibromyalgia is more common in those patients who are influenced by autoimmune diseases.[7] Maintaining the intensity of pain, increased physical activities and restoration of sleep/wake cycle have great significance in reducing the events of fibromyalgia.[8] The treatment and identification of all pain sources which are present in fibromyalgia are fundamental for the proper clinical management. The aim of the present study was to determine the level of various inflammatory markers in patients with fibromyalgia as compared to control individuals.

MATERIAL AND METHODS

A total of 50 patients of fibromyalgia diagnosed in the Department of Orthopaedics (Jinnah Hospital, Allama Iqbal Medical Collage, Lahore, Pakistan) and 50 healthy individuals were included in the study. None of the control individuals were on using multivitamins, cigarette or alcohol, nor did they have depression, liver cancer, diabetes mellitus or malnutrition syndrome. Research Ethical Committee of The Institute of Molecular Biology and Biotechnology approved the research protocol. Five ml of venous blood sample from each participant was taken from the anti-cubital vein. The sample tube was centrifuged within one hour of sample collection and serum was separated for storage at -70 °C until assayed.

Complete blood count of the participants was performed on automated haematology blood analyser (Sysmex version XP-2100). The levels of IL-1, IL-2, IL-6, IL-7, IL-8, IL-9, IL-17, IL-18, IL-1β and IL-1-RA were measured using commercially available kits (Glory Science Human Eliza Kits). MMP-2, 7, 9 were analysed with human diagnostic ELISA kit assay (Bio Compare).

The data was represented as mean and standard deviation. Independent sample *t*-test was applied to compare the data sets and $p<0.05$ was considered as statistically significant.

RESULTS

The data represented in Table-1 summarized the role of inflammatory markers in the patients with fibromyalgia. The mean values of IL-1, IL-2, IL-6, IL-7 and IL-9 were significantly increased in the patients with fibromyalgia when compared to control individuals. Elevated trends of IL-8, IL-17, IL-18, IL-1β and IL-1-RA were recorded in the patients with fibromyalgia as compared to control individuals respectively. The mean values of MMP-2, MMP-7 and MMP-9 were significantly increased in the patients with fibromyalgia as compared to control individuals.

Table-1: Interleukins and matrix metalloproteinases profile in fibromyalgia

VARIABLES	Control (n=50)	Subjects (n=50)	*p*
Interleukin-1 (pg/ml)	0.795±0.009	4.26±1.09	0.001
Interleukin-2 (pg/ml)	35.29±4.29	81.59±4.28	0.026
Interleukin-6 (pg/ml)	10.29±3.29	19.35±2.89	0.018
Interleukin-7 (pg/ml)	14.59±3.29	21.59±3.99	0.037
Interleukin-8 (pg/ml)	19.68±2.88	23.59±4.59	0.024
Interleukin-9 (pg/ml)	42.59±8.77	83.29±7.25	0.013
Interleukin-17 (pg/ml)	55.29±7.59	107.59±11.59	0.041
Interleukin-18 (pg/ml)	49.58±5.88	61.59±8.59	0.000
Interleukin-1β (pg/ml)	0.745±0.018	1.089±0.0095	0.032
Interleukin-1-RA (pg/ml)	3.290±0.956	4.59±1.113	0.015
Matrix metalloproteinases-2 (ng/ml)	423.25±10.59	625.32±18.59	0.038
Matrix metalloproteinases-7 (ng/ml)	49.58±4.28	85.59±10.25	0.000
Matrix metalloproteinases-9 (ng/ml)	41.59±8.59	101.25±10.11	0.011

DISCUSSION

The pro-inflammatory cytokines are considered to be the product of lymphocytes, which are stimulated not only by injury but also by neurons or glial cells. There are various stimuli that can induce this signalling cascade including TNF-α, IL-1, IL-18, IL-8, IL-2, IL-6, IL-17, IL-9, IL-6 and IL-1β. These pro-inflammatory cytokines act synergically and serve to mediate the formation of adhesion molecules through endothelial cells that are crucial for neutrophil recruitment at the site of inflammation. In the peripheral nerves[9,10], IL-1 alone or in combination with TNF-α enhances prostaglandin formation and stimulates substance P expression that reduces pain threshold. Patients with increased level of IL-1 may develop arthralgia, myalgia, headache and fever which can diminished by intake of COX inhibitors.[9]

Some authors observed IL-1-RA expression to be higher in patients with fibromyalgia and no difference was found between the concentration of IL-1 or IL-1-Rβ in these patients.[10] Higher expressions of TNF has been associated with rapid eye movement during sleep in these patients. In the present study, the

increased concentration of IL-8 was found in patients with fibromyalgia as compared to control individuals. This study is also consistent with the research work of Uceyler *et al*[11], who explained the role of IL-8 expressed in the plasma and serum of patients with fibromyalgia. The segregation of IL-8 is regulated by substance, which may lead to degranulation of tissue damage and neutrophil trafficking across vascular wall. In addition, the higher expressions of IL-8 induce sympathetic pain. In fibromyalgia, the upregulation of glial cells may be the consequences of intrathecal increase of cytokines and chemokines.[11] IL-8 levels were also overexpressed in cerebrospinal fluid, which was supported by glial cell activation by sympathetic activity.[11]

IL-6 is one of the most significant mediators for the stimulation of acute phase protein formation and secrete by hepatocytes during pain perception. IL-6 can be linked with depression stress, sympathetic nervous system (SNS) activation, fatigue and hyperalgesia. It is released by endothelial cells, glial cells and neurons. One of the most significant role of IL-6 in food intake, body weight and neurogenesis. Overexpression of IL-6 at the site of tender joints in response to the pain threshold is a significant characteristic for the progression of fibromyalgia.[12]

Aberrant expressions of CD4+ and Th17 have been associated with inflammatory and autoimmune diseases including lupus, psoriasis and rheumatoid arthritis. IL-17 is considered to be major cytokine secreted by Th17 cells and has significant role in the development of autoimmune and inflammatory diseases. The study of Pernambuoco *et al*[13] suggested the increased expression of IL-17 that has strong correlation with IL-10, IL-4, IL-2, IL-1, and IL-6 in these patients. IL-2 level has been associated to enhance cognitive impairment, myalgia, arthralgia and fatigue.[14]

The higher expressions of interleukins are controlled by various intracellular signalling pathways, from which IL-6 includes IL-6 receptor and gp-130 protein. IL-6 attaches to its receptor and signalling mechanism is triggered by suppressing Janus Kinases Signal and Activator of transcription (JAK/STAT) triggered transcription of TNF-α.[15] Some authors suggested that white adipose tissue stimulate the synthesis of IL-6 that cause low grade inflammation in the patients with fibromyalgia.

The patients with IL-2 LAK cell therapy for melanoma or renal cell carcinoma progress tender joints, cognitive impairment, arthralgias and myalgia. The fibromyalgia like symptoms appeared in those patients who were receiving interferon for chronic hepatitis. Brain cells have cytokine receptors and lymphocytes have opiate receptors that combine with substance P and mediate intracellular signalling cascade in these patients.[16] Neuro-immune cytokines have also been observed in these patients.

In the present study, the pro-inflammatory cytokines including IL-8, IL-1RA, IL-5, IL-2, IL-1 and IL-18 are considered to be important for inflammation, SNS, and hypothalamic pituitary axis. The SPS and HPA stimulation can also be facilitated by serotonin and acetylcholine that can be inhibited by opiates, γ-amino butyric acid, IL-2 and IL-6. In fibromyalgia, the sympathetic nervous system innervates immune cells including T-lymphocytes.[17] In addition, beta-agonist can reduce lymphocyte proliferative activity and natural killer cell response. IL-6 and IL-1 can separate beta-adrenergic receptors, IL-8 is stimulator of sympathetic pain and IL-6 can regulate sympathetic nervous system. Interestingly, IL-1 is synthesized by liver induced hyperalgesia through vagal afferents that can be inhibited via administration of IL-RA. In addition, it activates substance P gene expression and is strongly linked with anti-nociception and fever. In fibromyalgia, substance P regulates IL-8 expression that can be inhibited by IL-1RA. However, IL-8 is a pro-inflammatory cytokine that causes neutrophil trafficking around the vascular wall.[13]

TNF-α induces allodynia, pain inducing excitatory amino acids, rapid eye movement, and modulates substance P expression. In healthy individuals, IL-6 produce pain and fatigue, reduced cognitive activity, associates with depression, affects the hyperalgesia of corticosteroid and facilitates T-cell as well as B-cell proliferation. Substance P levels were overexpressed in the patients with fibromyalgia, which trigger the secretion of IL-6 levels.[18] IL-10 enhances B-cell concentrations, endorse energy through down-regulating type 1 activity and reduces TNF-α and IL-6 synthesis via monocytes. The psychological stress in patients with fibromyalgia is regulated through the synthesis of IL-6, IL-10, IL-1-RA and TNF-α. The patients with fibromyalgia have increased levels of IL-1-RA and gp-130 receptor as compared to control individuals.

Matrix Metalloproteinases (MMPs) are zinc dependent endopeptidases which damage extracellular matrix (ECM). MMPs are significantly involved in the plasticity, regeneration and development of central nervous system in the patients with fibromyalgia. In these patients, MMPs are abnormally expressed, cause damage to blood brain barrier, neuronal cell death, demyelination and infiltration of peripheral immune cells.[19] Expression of MMP-2 was higher in activated fibromyalgia and promotes intracellular signalling cascade including NF-ƘB, AP-1 and MAPK in these patients. MMP-8 serves to mediate inflammatory mechanism and expressed in various cell types at the site of inflammation including macrophages, epithelial choroid plexus, granulocytes, plasma cells and neutrophils.[20] The neuroinflammatory response of MMP-9 are strongly linked with TNF-α, which in turn cause tissue damage including diabetes, stroke,

ulcerative colitis, rheumatoid arthritis and fibromylagia.[21]

CONCLUSION

The present study concludes that the levels of inflammatory markers such as IL-1, IL-2, IL-6, IL-7, IL-8, IL-9, IL-17, IL-18, IL-1β and IL-1-RA were significantly higher among the fibromyalgia patients as compared to controls. It signifies the said inflammatory markers may have prominent role in the development and progression of disease condition.

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ORIGINAL ARTICLE

FREQUENCY OF MICROALBUMINURIA IN HYPERTENSIVE PATIENTS WITH ACUTE ISCHEMIC STROKE

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Background: Stroke, and its most common variant ischemic stroke, is one of the major causes of morbidity and mortality worldwide. Besides conventional risk factors, microalbuminuria (MA) is considered to be associated with atherosclerosis, hence ischemic stroke. Early detection of microalbuminuria may help in identifying hypertensive patients at high risk of ischemic stroke. The aim of current study was to determine the frequency of microalbuminuria in hypertensive patients with ischemic stroke. **Methodology:** This cross-sectional study was conducted at the Department of Medicine, Hayatabad Medical Complex Peshawar, Pakistan from July 2020 to June 2021. A total of 196 hypertensive patients of either sex with ischemic stroke were included in this study. They were tested for microalbuminuria in a 24-hours urine sample using Pyrogallol Red method. Urine albumin concentration of 30–300 mg/day was considered as microalbuminuria. The frequency of microalbuminuria was stratified according to age and gender using Microsoft Excel sheet. **Results:** The participants were aged 30–70 years and most of them (88, 45%) were in the age group 61–70 years. Majority (122, 62%) of the patients were male. Microalbuminuria was identified in 92 (47%) patients with slight male predominance (57, 62%). **Conclusion:** Given the high frequency of Microalbuminuria in hypertensive patients with acute ischemic stroke, microalbuminuria is a useful and inexpensive parameter in identifying hypertensive patients at increased risk of ischemic stroke.

Keywords: Albuminuria, Hypertension, Diabetes mellitus, Ischemic stroke

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INTRODUCTION

Stroke or cerebrovascular accident (CVA) is a focal neurological deficit due to vascular lesion. It is sub-classified into ischemic and haemorrhagic CVA. The former accounts for more than 80% cases worldwide.[1] Ischemic stroke is a major cause of mental and physical disability making it the third leading cause of death in the developed countries.[2] According to 2015 WHO report, nearly 5.7 million deaths were caused by stroke and over 50% deaths occurred in Asia.[3] The reported prevalence of stroke is 4.8% in Pakistani population.[4]

Stroke has risk factors that are related to coronary heart disease and other cardiovascular illnesses. Targeting the primary modifiable causes of hypertension, high triglycerides, dyslipidaemias and diabetes are all effective preventative measures. Lifestyle-related risks can also be included. Smoking, lack of physical activity, unhealthy diet, and abdominal obesity are all factors that contribute to cerebrovascular events. Major important modifiable risk factors for stroke are diabetes mellitus, hypertension, and smoking. Other risk factors include age above 55 years, heavy alcohol consumption, left ventricular hypertrophy, arrhythmias and illicit drugs use.[5] Atherosclerosis of the cerebral arteries is believed to be the main aetiological factor in ischemic stroke, and hypertension is one of the major risk factors for cerebral atherosclerosis.[6,7] Microalbuminuria (MA), defined as albumin concentration of 30–300 mg/day in a 24-hour urine collection, is considered to be associated with atherosclerosis and arterial hypertension.[8,9] It is considered to be a marker of vascular endothelial damage due to the severity and/or duration of various pathophysiological insults especially poorly controlled diabetes mellitus and hypertension.[10] Albumin leakage into the urine may reflect the renal sign of global endothelial dysfunction, and has been associated with an elevated risk of serious cardiovascular events including stroke.[11]

The pathophysiologic processes which link MA and cerebrovascular diseases are unclear. The suggested mechanism of microalbuminuria resulting in clinical vascular disease is increased systemic vascular permeability due to endothelial dysfunction caused by systemic atheroma formation.[12] It is believed to be an independent risk factor for acute ischemic stroke as well as cardiovascular diseases.[13] MA is strongly linked to stroke risk and its measurement is one of the most important screening test for determining the risk of atherosclerotic disease as well as those who are prone to develop ischemic stroke.[14] The aim of this study was to determine the frequency of MA in hypertensive patients with acute ischemic stroke.

METHODOLOGY

This cross-sectional descriptive study was carried out at the Department of Medicine, Hayatabad Medical Complex (HMC), Peshawar, Pakistan, from July 2020

to June 2021. Ethical approval was obtained from the Departmental Committee and patients were enrolled after written informed consent from the patients or their relative if the patient was unable to give consent.

A total of 196 hypertensive patients admitted to the Medical Unit, with a diagnosis of acute ischemic stroke on the basis of focal neurological deficit and infarction on CT scan or MRI brain within 72 hours of admission, were included in the study with non-probability consecutive sampling technique[15], with 95% confidence interval and 7% margin of error. Sample size was calculated using WHO sample size calculator[16]. Patients were divided into 4 groups based on age: Group I (30–40 years), Group II (41–50 years), Group III (51–60 years), and Group IV (61–70 years).

The diagnosis of hypertension was confirmed on measuring blood pressure twice by mercury sphygmomanometer in all cases. After confirming hypertension, the evaluation of microalbuminuria was made on 24 hours collection of urine testing with Pyrogallol Red method in the Biochemistry Section of the Main Laboratory of HMC, Peshawar.

Data was recorded on a specially designed proforma for all numerical and categorical variables including age, gender, hypertension, and microalbuminuria. Statistical analyses were performed on IBM SPSS-20. Simple arithmetic analyses (mean, standard deviation and/or percentages) were deduced for each parameter. Categorical variables were described in terms of frequencies and percentages.

RESULTS

Participants of the current study were analyzed based on age distribution and they ranged in age from 30–70 years. Group I had 20 (10%) patients, Group II had 29 (15%) patients, Group III had 59 (30%) patients and Group IV comprised of 88 (45%) patients. Minimum duration of stroke was 3 hours while maximum duration since stroke was 70 hours. Mean age of the patients was 60±2.71 Years (Table-1).

Out of the 196 patients under study, 122 (62%) were male while 74 (38%) were female (Table-2). Microalbuminuria was observed in 92 (47%), whereas 104 (53%) patients did not have microalbuminuria. Stratification of microalbuminuria with age and gender is shown in Table-3 and 4 respectively.

Table-1: Age distribution (n=196)

Age Groups	Mean Age (Years)	Frequency	Percentage
Group I (30–40 Years)	60±2.71	20	10
Group II (41–50 Years)		29	15
Group III (51–60 Years)		59	30
Group IV (61–70 Years)		88	45

Table-2: Gender distribution of patients (n=196)

Gender Distribution	Frequency	Percentage
Male	122	62
Female	74	38

Table-3: Stratification of microalbuminuria with age (n=196)

Micro-albuminuria	Group I	Group II	Group III	Group IV	Total
Yes	9	14	28	41	92 (47%)
No	11	15	31	47	104 (53%)
Total	20	29	59	88	196 (100%)

χ^2 test applied, (p=0.996, NS)

Table-4: Stratification of microalbuminuria with gender (n=196)

Microalbuminuria	Male	Female	Total
Yes	57	35	92
No	65	39	104
Total	122	74	196

χ^2 test applied, (p=0.94, NS)

DISCUSSION

Cerebrovascular accident (CVA) and its most common variant, ischemic stroke has a devastating multifaceted effect on an individual's life. It has got some risk factors and work has been done nationally and internationally to study these risk factors. Along with the conventional risk factors, microalbuminuria has been considered as a new potential risk factor for ischemic stroke. The current study was conducted to elucidate the frequency of MA in hypertensive patients with acute ischemic stroke, as literature lacks studies depicting this frequency in local population. In this study, majority of the patients were male 122 (62%) and most of them (88, 44.90%) were in the age group 61–70 years; mean age was 60±2.71 years. These findings are supported by those of a study conducted on 120 ischemic stroke patients in Bangladesh which identified male predominance and higher mean age.[17]

The results of the current study revealed that MA is present in 47% hypertensive patients who presented with acute ischemic stroke, whereas 53% were not having MA. These findings are in accordance with those of a previous study conducted on 60 Polish ischemic stroke patients, which identified MA to be present in 46.7% of acute non-diabetic ischemic stroke patients.[18]The close relation between MA, atherosclerosis, hypertension and stroke suggest that microalbuminuria is a potentially inexpensive, and easily measured marker of increased risk for ischemic stroke.[19]

Presence of MA in hypertensive patients indicates the initial stages of atherosclerosis which is alarming for stroke. Early detection of MA will help in identifying hypertensive patients at high risk of ischemic stroke. Timely pharmacological intervention with angiotensin converting enzyme inhibitors (ACEIs), angiotensin receptor blockers (ARBs), calcium channel blockers (CCBs), β-blockers, Aliskiren, aldosterone antagonists and statins may help to decrease morbidity and mortality in this group.[20] However, other factors like obesity, dyslipidaemias, atrial fibrillation, ischemic heart

disease, left ventricular hypertrophy, hypercoagulable states (myeloproliferative disorders, protein C and S deficiency, high factor VIII level, antiphospholipid syndrome) family history of ischemic stroke, metabolic syndrome, inherited conditions of kidney are also important to be addressed as risk factors for cerebrovascular problems.

CONCLUSION

Given the high frequency of Microalbuminuria in hypertensive patients with acute ischemic stroke, microalbuminuria is a useful and inexpensive parameter in identifying hypertensive patients at increased risk of ischemic stroke.

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ORIGINAL ARTICLE

EFFECTS OF AVOCADO (*PERSEA AMERICANA*) AQUEOUS SEED EXTRACT ON GROSS FEATURES OF LIVER AND HEPATOCYTE SIZE IN ISONIAZID AFFECTED ALBINO RATS

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Background: Isoniazid (INH) has been associated with severe hepatotoxicity and fatal liver injury. This study aimed to observe the hepatoprotective effects of concomitant use of avocado aqueous seed extract on the gross features of liver and size of hepatocytes in INH induced hepatotoxicity in albino rats. **Methods:** This experimental study was conducted from Jan–Jun 2019. Thirty-six male albino rats were divided into 4 groups. Groups were administered treatment orally for 30 days. Group 1 received distilled water 1 ml/Kg/day. Group 2 received INH 100 mg/Kg/day dissolved in 1 ml distilled water. Group 3 was given aqueous avocado seed extract 250 mg/Kg/day in 2 ml distilled water and Group 4 received aqueous avocado seed extract 500 mg/Kg/day in 4 ml distilled water. **Results:** Highest mean liver weight was seen in Group 4 and lowest in Group 2. The weight of liver was significantly different among all groups but Relative Tissue Weight Index among all groups was insignificant. Colour of liver in four groups varied from reddish to pale red and reddish brown. The texture of all livers was smooth. Hepatocyte size was found to be largest in Group 2 and smallest in Group 1. Comparison among groups showed significant difference in sizes of hepatocytes. **Conclusion:** Concomitant use of aqueous avocado seed extract prevents INH-induced hepatotoxicity by maintaining body and liver weights, gross features of liver, and size of hepatocytes in a dose-dependent manner.

Keywords: Avocado, Isoniazid, Liver, Hepatocytes, Albino rats

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INTRODUCTION

Isoniazid (INH) has been used as a first line drug for the treatment of TB since 1952.[1,2] Structurally, it has a pyridine ring and hydrazine group and has potent bactericidal effects on rapidly growing TB bacilli.[1] INH has been associated with severe hepatotoxicity and fatal liver injury by causing necrosis and steatosis of hepatocytes.[3]

The incidence of liver toxicity with isoniazid is 1.6%. Metabolites of INH mainly produce oxidative stress resulting in hepatic injury.[4,5] Oxidative stress is caused by depletion of both bound and free glutathione (GSH), increase in the activity of superoxide dismutase which readily converts O_2 into hydrogen peroxide (H_2O_2), decrease in the catalases, and glutathione peroxidases that readily remove H_2O_2 resulting in buildup and accumulation of H_2O_2.[5,6] Hydrogen peroxide is a very potent oxidizing agent causing oxidative stress either directly or indirectly through the generation of reactive oxygen species (ROS).[6]

Oxidative stress is the main reason of INH induced hepatotoxicity and substances that have anti-oxidant properties can prevent it. Avocado is one of such possible fruits that can decrease the INH induced hepatotoxicity. Avocados are large berries containing a single seed weighing about 12–16% of the total weight of the fruit.[7] Avocado is a rich source of glutathione. Each 100 grams contains 27.7 mg of glutathione, making it the richest source, secondary to asparagus.[8] Glutathione is referred to as body's master antioxidant. The anti-oxidant effects of avocado are also because of the fact that it increases the serum glutathione levels as reported by Mahmoed *et al*.[9]

Ultrasonic extractions of avocado seed and peel demonstrated high contents of phenolic compounds in both ethanolic and aqueous seed and peel extracts than that of pulp.[10–12] Avocado seeds contain 64% of total phenolic compounds present in it and are responsible for 57% of total antioxidant capacity.[13]

The objective of the present study was to observe the hepatotoxic effects of concomitant use of Avocado (*Persea americana*) aqueous seed extract on the gross features of liver and size of hepatocytes in Isoniazid (INH) induced hepatotoxicity in albino rats.

METHODOLOGY

It was an Experimental study conducted on 36 adult male albino rats after taking approval from the IRB (letter# 205/RC/KEMU) and ASRB (letter# 10220/ KEMU/2018), KEMU. The study was carried out from Jan to Jun 2019 at Experimental Research Laboratory (Animal House) of Postgraduate Medical Institute, Lahore in collaboration with Anatomy Department and Histopathology Laboratory of KEMU.

A total of 36 male Sprague Dawley Albino rats of 8–12 weeks age, weighing between 200–250 grams were randomly divided into 4 equal groups by lottery method. Animals were allowed to acclimatize for one week before start of experiment. Any rats that became inactive or stopped eating were excluded. They were kept under controlled conditions of temperature (27–30 °C) and 12 hours of light and dark cycle were maintained. The animals were fed on standard diet and tap water *ad libitum*.

Group 1, (Control group, CG) received only distilled water 1 ml/Kg/day in morning.[14] Group 2, (Isoniazid group, INHG) received only isoniazid 100 mg/Kg/day dissolved in 1 ml distilled water as a single dose in morning.[4] Group 3, (Isoniazid-Avocado (low dose) group, $INHAV_{low}$) was given Isoniazid 100 mg/Kg/day dissolved in 1 ml distilled water as a single dose in morning[4], and Avocado seed extract (aqueous) 250 mg/Kg/day dissolved in 2 ml distilled water as a single dose in morning, one hour after INH.[14] Group 4, (Isoniazid-Avocado (high dose) group, $INHAV_{high}$) received Isoniazid 100 mg/Kg/day dissolved in 1 ml distilled water as a single dose in morning[4], and Avocado seed extract (aqueous) 500 mg/Kg/day dissolved in 4 ml distilled water in two divided doses of 2 ml each. 1st dose was given in morning, one hour after INH and 2nd dose in the afternoon. All doses were given orally by gavage method for 30 days.

The body weights of experimental animals were measured at day zero, on weekly basis for dose adjustment, and at the end of experiment on 31st day. After 24 hours of the last dose of drugs animals were euthanized with morphine 0.3 to 0.5 mg/Kg intraperitoneally[15] and liver was dissected out. It was washed with normal saline and weighed. Gross examination of liver was done and photographed.

The liver specimens were fixed in 10% formalin solution for processing. The slides were stained with Haematoxylin and Eosin (H and E) stains for microscopy. The slides were examined for hepatocytes at 20× magnification using Nikon Eclipse Ci-L LED microscope. In each slide, 3 fields were studied and 5 hepatocytes with clearly demarcated cell membranes were selected. Average of hepatocyte dimensions were taken as size of that hepatocyte. Mean size of 15 hepatocytes was taken as size of hepatocyte for that particular slide. Mean size of hepatocytes of 3 slides was taken as hepatocyte size for that particular specimen.

The data from the 4 groups was entered and analysed using SPSS-26. Mean±SD were calculated for quantitative features. One-way ANOVA was used to construct the comparison among all groups. Qualitative or categorical data were presented as frequency and percentage. Pair-wise comparison was done using Least Square Difference (LSD) Test. Chi-square test was used to construct a comparison among groups on the basis of qualitative/categorical characteristics, and $p<0.05$ was taken as significant.

RESULTS

The Mean±SD of initial body weights of rats of four groups were 200.4±9.71 g (CG), 203.89±29.41 g (INHG), 200.44±14.98 g ($INHAV_{low}$) and 210.56±24.58 g ($INHAV_{high}$). The final body weights were 223.56±8.09 g (CG), 185.11±20.55 g (INHG), 197.56±22.86 g ($INHAV_{low}$), and 248.33±23.85 g ($INHAV_{high}$). An increase in final body weight in CG and $INHAV_{high}$, but a decrease in final body weight in INHG and $INHAV_{low}$ was observed. Comparison among groups for initial and final body weights by One-way ANOVA showed that the difference of final body weight among all groups was significant ($p=0.000$). However, pair-wise comparison among groups for final body weight showed significant difference in all possible groups ($p<0.05$) except between groups INHG and $INHAV_{low}$.

Mean liver weight of the rats of four groups were 7.28±0.96 g (CG), 6.69±0.88 g (INHG), 6.78±0.76 g ($INHAV_{low}$) and 8.32±1.09 g ($INHAV_{high}$). Highest mean liver weight was seen in $INHAV_{high}$ and lowest in INHG. Relative Tissue Weight Index (RTWI) was 3.26±0.43 (CG), 3.61±0.29 (INHG), 3.44±0.18 ($INHAV_{low}$) and 3.35±0.28 ($INHAV_{high}$). One-way ANOVA showed that the weight of liver was significantly different among all groups ($p<0.05$) but RTWI among all groups was insignificant (Table-1). When pair-wise comparison was done using LSD test, it showed that weight of liver in $INHAV_{high}$ is significantly more than that of other groups ($p<0.05$). RTWI was significantly higher in INHG as compared to CG but in all other possible pairs between groups, it was insignificant. (Table-1).

Table-1: Comparison of final body weights, weight of liver, and RTWI (One-way ANOVA) among groups

	Sum of Squares	df	Mean Square	F	*p*
Body Weight Final (g)					
Between Groups	21370.97	3	7123.66	18.04	0.000*
Within Groups	12639.33	32	394.98		
Total	34010.31	35			
Weight of Liver (gram)					
Between Groups	15.184	3	5.061	5.831	0.003
Within Groups	27.776	32	0.868		
Total	42.960	35			
Relative Tissue Weight Index					
Between Groups	0.617	3	0.206	2.166	0.111
Within Groups	3.039	32	0.095		
Total	3.656	35			

Colour of liver in 4 groups varied from reddish to pale red and reddish brown. In CG, it was reddish in

all animals (Figure-1). However, in INHG, 33.3% had reddish and 66.7% had pale red colour (Figure-2) and in $INHAV_{low}$ group, 66.7% had reddish, 11.1% had pale red and 22.2% had reddish brown colour. Reddish colour of the livers was seen in 77.8% $INHAV_{high}$ group animals, and 22.2% had reddish brown colour. Colour change was statistically significant in all experimental groups (p=0.002).

The texture of all livers was smooth. No other gross abnormality except haemorrhagic spots was observed. In INHG, 33.3% livers, and in $INHAV_{low}$ group 22.2% livers had haemorrhagic spots.

The slides showed hexagonal shaped hepatic lobules, each having central vein lined by squamous epithelium, one or two cell thick radiating cords of hepatocytes and peripherally arranged portal triads. Hepatocytes were polygonal in shape in all groups. Mean sizes of hepatocytes for four groups were 18.93±1.07 µm (CG), 28.69±1.45 µm (INHG), 26.71±1.52 µm ($INHAV_{low}$) and 20.52±1.57 µm ($INHAV_{High}$). Hepatocyte size was found to be largest in INHG and smallest in CG. Comparison among groups by One Way ANOVA showed significant difference in sizes of hepatocytes (p=0.000). LSD test showed a significant increase in sizes of hepatocytes in INHG, $INHAV_{low}$ and $INHAV_{High}$ in comparison to CG (p<0.05). When $INHAV_{low}$ group was compared with INHG, a statistically significant decrease in sizes of hepatocytes was observed (p=0.006). However, the test also showed that the hepatocytes of $INHAV_{High}$ group were significantly smaller in size as compared to INHG and $INHAV_{low}$ (p=0.000). (Table-2).

Table-2: Comparison among groups for size of Hepatocytes (µm) (One Way ANOVA)

Size of Hepatocytes (µm)	Sum of Squares	df	Mean Square	F	*p*
Between Groups	601.375	3	200.458	99.948	0.000*
Within Groups	64.180	32	2.006		
Total	665.556	35			

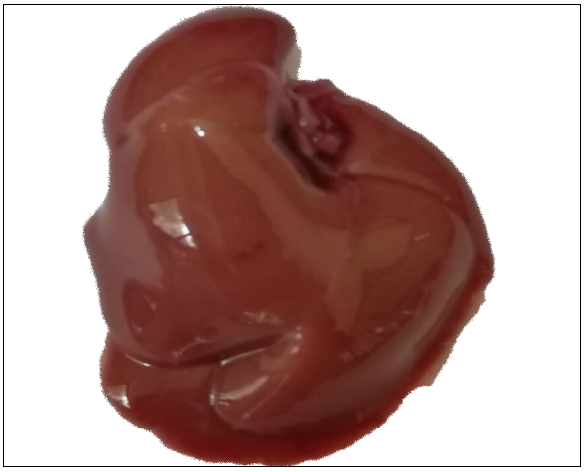

Figure-1: Reddish liver of control group

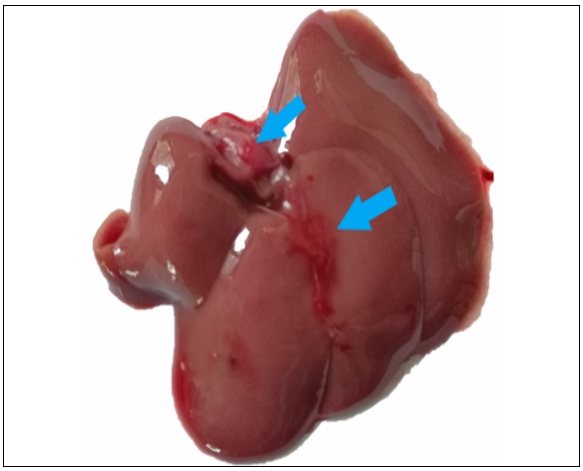

Figure-2: Pale red liver with haemorrhagic spots

DISCUSSION

In the current study, variations were observed in final body weights when the weights of experimental animals were measured at the start and end of experiment were statistically analysed. In control group, the mean weight increased, while in the INHG a decrease in body weight was seen. This effect was in accordance with a previous study conducted by Cavanagh[16] on a rat model where INH was given in a dose of 250 mg/Kg/day by oral and subcutaneous routes. It concluded that oral INH produced failure to gain weight because of more pronounced absorptive inhibitory effects on digestive system than subcutaneous route in absorption of amino acids through intestinal wall.[16] In the group that received avocado seed extract in low dose along with INH ($INHAV_{low}$), the final body weight was also found to be reduced but this decrease in weight was less than that seen in INHG. It could most likely be due to co-administration of avocado seed extract. The experimental animals which were administered high dose of extract along with INH ($INHAV_{high}$) showed a significant increase in final body weight as compared to other groups. This indicated an effective dose dependent role of avocado seed extract on weight gain even in presence of INH. This finding is in concordance to the observations of Alhassan *et al*[17]. They observed the effects of avocado seed extract on diabetic rats. There was a significant increase in body weight of rats in comparison to those which did not receive extract. It was probably because the extract overcame the INH induced inhibitory effects on intestinal amino acid absorption, thus enhancing proper utilization of nutrients.[17] This corresponds to the results of our study in terms of weight gain seen in $INHAV_{high}$.

The weight of the liver was in accordance with the final body weight of rats in all groups when the mean liver weight and RTWI for each liver was calculated. However, mean liver weight was lowest in INHG and it was noted that weight of the liver in

$INHAV_{high}$ was significantly higher in comparison to other groups. The reduced liver weight in INHG is in agreement to the finding of Humayun *et al*[18] who studied the hepatoprotective effects of propolis ethanolic extract in INH induced hepatotoxicity in albino mice.[18] The gain in liver weight with high dose of avocado seed extract in the present study was similar to the results observed by Uchenna *et al* who studied the effects of avocado ground seeds on carbohydrate and lipid metabolism in a rat model. An increase in weight of liver was documented on giving diet containing 8% of avocado seeds.[19]

The texture of the liver was found to be smooth showing no deleterious effects of INH on liver surface as reported by Humayun *et al*[20]. In the present study, variations of colour were observed. In CG, all livers were reddish in colour reflecting good vascularity of tissue. In INHG, most of the livers were pale red and a few had haemorrhagic spots. This pale colour change was also reported by Enriquez-Cortina *et al*[21] who studied the hepatoprotective effects of hepatocyte growth factor (HGF) on INH and Rifampicin (RIF) induced hepatotoxicity on mice. The explanation given by Groossman for this effect was because of INH induced oxidative stress, there was decreased liver vascularity due to vasoconstriction secondary to thickening of tunica media of blood vessels by oxidative stress induced smooth muscle cell proliferation and collagen deposition.[22] In the present study, in $INHAV_{low}$ group, the colour of liver was found to be reddish in most rats and a few haemorrhagic spots were observed. In $INHAV_{High}$ group, the frequency of healthy reddish coloured livers was more than $INHAV_{low}$ indicating the possible role of avocado seed extract in maintaining liver vascularity. Two animals of each of $INHAV_{low}$ and $INHAV_{High}$ groups showed reddish brown liver colour.

The size of hepatocytes was found to be smallest in the CG and largest in INHG. Similar results were reported by Humayun *et al* who studied the changes in liver histology produced by INH in mice.[20] Liu *et al* also reported increase in size of hepatocytes after 30 day treatment with ATT drugs.[23]

According to Simon *et al*[24], INH induced oxidative stress is believed to disturb the Na^+ equilibrium across cell membrane mainly affecting Na^+ efflux, ultimately resulting in Na^+ retention which leads to cellular swelling and death. The same mechanism might have been responsible for this observation in the current study. A significant dose-dependent decrease in size of hepatocytes was observed in $INHAV_{low}$ and $INHAV_{High}$ groups as compared to INHG. This was probably due to anti-oxidant effects exerted by avocado seed extract to reduce cellular swelling by inhibiting oxidative stress. Similar results were reported by Hamidian *et al*[25]. They investigated the effects of methanolic avocado seed extract on liver of diabetic mice and at the end of the experiment, the results indicated an increase in size of hepatocytes in diabetic mice.

CONCLUSION

The concomitant use of aqueous avocado seed extract helps to prevent the INH-induced decrease in body and liver weights in a dose-dependent manner. It is also beneficial in maintaining the gross features of liver and size of hepatocytes in INH affected albino rats.

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GM: Revision of manuscript
SI: Data analysis

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ORIGINAL ARTICLE

CLINICAL PROFILING AND OUTCOME OF ACUTE POST-STREPTOCOCCAL GLOMERULONEPHRITIS IN CHILDREN FROM A TERTIARY CARE CENTRE

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Background: Childhood throat and skin infection caused by group A beta haemolytic streptoccus is very common and is associated with many complications. Objectives of this study were to document the clinical profile of children with Acute Post-Streptococcal Glomerulonephritis. **Methods:** This cross-sectional study was conducted in Paediatrics Department of Ayub Teaching Hospital Abbottabad from March 2019 to March 2020. Total 56 patients fulfilling inclusion criteria were included in the study, data was recorded and analysed on SPSS-20. Descriptives recorded and *p*-values calculated. **Results:** APSGN was common (53.57%) in 7–11 years age group with a peak at 9 years. It was more common in males (62.5%). Minimum age was 3 years and maximum was 16 years, mean age being 8.72±2.762. Preceding sore throat triggered immune response in 33.9% in comparison with past streptococcal skin infection in 19.7%. ASO titres were raised in 42.9%. Hypertension was most common presentation affecting 96.4% patients followed by oedema in 67.9%. Atypical presentations of seizures and hypertensive encephalopathy were observed in 28.6%. Gross haematuria and microscopic haematuria occurred in 41.1% and 42.9% patients respectively. Majority (87.5%) did not develop acute kidney failure. **Conclusion:** APSGN in children has a male predominance. Sore throat as antecedent infection affects older children and is more common as compared to skin infection in triggering this immune response. Atypical manifestations, cardiac and neurological complications including emergencies such as status epilepticus should be watched for.

Keywords: Acute nephritic syndrome, Post infectious glomerulonephritis, Post streptococcal glomerulonephritis

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INTRODUCTION

Glomerulonephritis is the term reserved for the variety of renal disease in which inflammation of the glomerulus, manifested by proliferation of cellular elements, is secondary to immunologic mechanism.[1,2] Group A streptococcus (GAS) is one of the most common infection in children leading to a wide spectrum of diseases, ranging from the common skin and throat infections to invasive diseases and post infectious sequelae including glomerulonephritis.[3] It has been noted that over 470,000 cases of Acute Post-Streptococcal Glomerulonephritis were seen every year worldwide, out of this 97% occurring in developed countries and approximately 5000 cases (1% of total cases) resulting in death.[4]

Acute Post-Streptococcal Glomerulonephritis (APSGN) is characterized by classic triad of, rapid onset of gross hematuria, oedema, and hypertension and is usually secondary to episode of GAS pharyngitis or pyoderma.[5] Serologic evidence of a recent streptococcal infection should be sought in suspected cases of APSGN because positive streptococcal serology are more sensitive (94.6%) than history of recent infection (75.7%) or positive cultures (24.3%) in supporting the diagnosis.[6] This study aims to find and document the clinical presentations, disease profile in children presenting with APSGN. Resulting findings are discussed in detail in reference to studies in other developing countries.

METHODOLOGY

This cross sectional study was undertaken in department of paediatrics at Ayub teaching hospital Abbottabad from March 2019 to March 2020. After ethical approval; data was collected on proforma from patients fulfilling the inclusion criteria. This included patients of either gender and within ages of 3 to16 years, with clinical profile of hematuria, hypertension, oedema, presence of RBC casts in urine, raised ASO titres & hypertension. All patients of chronic kidney disease (CKD), urinary calculi, congenital urinary tract abnormalities and connective tissue disorder were excluded. Clinically suspected APSGN or nephritic syndrome but not proven on investigation as APSGN were also excluded. The data was analysed using SPSS-20. Descriptives were calculated and χ^2 test applied; $p \leq 0.05$ was considered statistically significant.

RESULTS

Of the 56 patients, 35 (62.5%) were male and 21 (37.5%) were female. Mean age was 8.72±2.762 Years

(Range 3–16 Years). APSGN mostly (53.57%) affected children in age range 7–11 years with a peak at 9 years (Table-1).

Table-1: Age distribution of patients

Age group	Number	Percentage
3–6 Years	15	26.79
7–11 Years	30	53.57
12–16 Years	11	19.64

Sore throat affected 33.9% children and was more common in the older age group of 10–12 year, whereas skin infection affected considerably lesser patients, i.e., a total of 19.7% but was a precipitating cause for APSGN in the younger age group of 6 years age. From the total only 24 (42.9%) had raised ASO titres. A history of preceding or accompanying fever was recorded in 35.7% of cases. Hypertension was the most common clinical presentation present in 54 (96.4%) of patients, after hypertension, oedema was the second commonest manifestation and was observed in 38 (67.9%) of patients. Atypical manifestations such as seizures and hypertensive encephalopathy were commonly observed in nearly 16 (28.6%) of patients. Other cerebral complications such as visual loss were less common and seen in only 3.6%. In renal manifestations, 23 patients presented with gross haematuria and 21 with microscopic haematuria. Majority of the patients did not develop acute kidney failure as a complication so it was a lesser known complication in our study. Cardiopulmonary manifestations such as cardiomegaly, shortness of breath were present in between 8 to12% of patients, and pulmonary oedema in 3.6% only. Casts were seen in urine in 66% and pus cells in 57.1 % but only 3.6% had dysuria. (Table-2).

Table-2: Descriptive Statistics (n=56)

Clinical presentations/ Labs	Observation	N	%	*p*
Sore throat	Present	19	33.9	0.462
Skin infection	Present	11	19.7	0.471
Hypertension	Present	54	96.4	0.270
CCF	Present	7	12.5	0.142
Plain chest X-ray findings	Normal	46	82.1	0.381
	Cardiomegaly	7	12.5	
	Pulmonary oedema	2	3.6	
	Bronchopneumonia	1	1.8	
ASO titre	Positive	24	42.9	0.403
Oedema	Present	38	67.9	0.33
Facial swelling	Present	16	28.6	0.148
Fever	Present	20	35.7	0.222
Headache	Present	8	14.3	0.240
Vomiting	Present	7	12.5	0.042
Shortness of breath in days	Present	5	8.9	0.039
Cola coloured urine (gross haematuria)	Present	19	33.9	0.18
Status epilepticus	Present	7	12.5	0.004
Seizures	Present	16	28.6	0.010
Hypertensive encephalopathy	Present	11	19.6	0.184
Unconsciousness	Present	8	14.3	0.120
Hemianopia/visual loss	Present	2	3.6	0.140
Dysuria	Present	2	3.6	0.347
Oliguria	Present	1	1.8	0.325
Gross haematuria	Present	23	41.1	0.168
Microscopic haematuria	Present	24	42.9	0.131
Cast in urine	Present	37	66.1	0.164
C3 levels low	Present	11	-	-
Pus cells in urine	1–20 pus cells in urine	32	57.1	
	21–40 pus cells in urine	2	3.6	
	Numerous	7	12.5	
	Missing	14	25.0	
Acute renal failure	Absent	49	87.5	0.142
	Couldn't establish	7	12.5	

DISCUSSION

Patients in the present study were 7–11 years old with peak at 9 years. Agarwalla SK observed not only more cases in same age groups but a male preponderance as well.[7] In a Bangladesh study by Akiteruzzaman M, similar age groups were recorded[8], whereas in Nepal most common age of presentation for APSGN was 9 years.[9] Tejani *et al.* also observed APSGN affecting age group between 2–10 years with only less than 5% being below the age of 2 years.[10] In a South Indian study mean age recorded was recorded at 6.8.[11] Reason that APSGN is rare in the very young children because of their immature immune response and since it is an autoimmune condition so requires a fully responsive immune system that is yet to develop in young child.[12,13]

APSGN is known to affect males more commonly as compared to females. In a study done in Bangladesh, this ratio was found to be 2.4:1.[8] Agarwalla SK found that males were affected 1.6 times more as compared to females.[7] The cause for this male predominance isn't clear.[14]

Fever was recorded in 35.7% of patients and a preceding history of sore throat was a more common complaint affecting 33.9% as compared to a history of skin infection which was recorded only in 19.7% children. Sore throat as a preceding infection was most common in the older age group 12 years, whereas skin infection as the precipitating factor mostly affected the 6 years old. Bhalla K *et al* recorded sore throat to be the commoner antecedent factor affecting 70.83% compared to skin infection present in 10.42% of cases. The age groups also matched with ours as it was also noted by Bhalla *et al* that pharyngitis less common in the younger children.[15]

Oedema affected 67.9% children and this presentation was less common in our study than studies done in India and Nepal and India (97.97.5% and 83.4% respectively)[9,11] and was more in accordance with an Indonesian study affecting 76.3% children.[16]

ASO titres were recorded high in 42.9% cases. ASO rising titres are helpful in aiding diagnosis of recent streptococcal infection but may not be so helpful in skin infection by streptococcus as adipose tissue in the skin may be acting as a barrier.[17] Interestingly among atypical manifestations, neurological complications were common in our setup and acute kidney injury was less common outcome.

Malla K *et al* also recorded hypertensive encephalopathy in 18.6% patients, our study showed even a higher percentage (19.6%).[18] In our study hypertension was recorded in 54 patients and led to seizures and hypertensive encephalopathy in 19.6% of patients. Two patients had to undergo a CT scan of the brain that showed cerebral ischemia. In two other studies hypertensive encephalopathy was a much lesser presentation observed in 5% and 4.3% of cases respectively.[19,20]

Agarwalla *et al* recorded hypertension in 90% patients and cerebral complications in 35% of patients.[7] Accompanying cerebral manifestations in our study remained upto14%. In the Bangladesh study the atypical presentation of hypertensive encephalopathy was seen in only 8.8%[8], and even lower at 4.6% by Gunshekran *et al* study.[11]

Gross and microscopic hematuria occurred in 41.1% and 42.9% respectively. Akterruzeman K *et al*, recorded microscopic hematuria in 100% of the patients but presence of hematuria might not be an indicator of severity of disease.[8] Casts in urine were found in 66.1% of our patients .This Higher percentage was present in studies by Travis and Kalian (60–85%), also found in USA and Nigeria (80%) and (65%) respectively.[21–23] This was a less common finding in the Bangladesh study, i.e., 45%[8], and was a less common occurrence in studies done in Indonesia, and India (44.3% and 37.1%) respectively.[16,24] Agarwalla SK found serum C3 level to be low in 83% of the cases[7], but as it's done in private lab we could perform only in a few patients, a low level was found in 11 and a percentage can't be quoted. Other renal complications like acute kidney injury and nephrotic range proteinuria couldn't be established in any patient. Studies done elsewhere found Azotemia to be higher (47.8%)[8,9] and even up to 80% in Iran (80%), but low in Indonesia, where it occurred in only 10% cases.[25,26]

Likewise cardiac complications were less common as compared to studies done elsewhere. Breathlessness and cardiomegaly were noted between 10–12.5% cases while pulmonary oedema was present in only 2 (3.6%) cases, with bronchopneumonia in only one case. Pleural effusion in our study was similar to Manhas *et al* (3%).[24] Pleural effusion with other radiological abnormalities were very high in studies done by Puri *et al* (72%) and Kirckpatrick *et al* (85.5%).[26,27] Albar and Rauf recorded (81.6%) radiological abnormalities due to pleural effusion and other abnormalities such as pulmonary oedema and pneumonia. Chest X-ray was normal in 82.1% cases in our study.[16,26,27]

Malla K quoted that Heart failure and acute renal failure were the sole systemic complications in 7 out of 29 and 2 out of 29 APSGN patients respectively.[18] This was also noted by Olowa WA in Nigeria.[28] Heart failure was seen in 3% cases in another study[29], whereas in our study only 10% presented with shortness of breath, cough, and hepatomegaly. Acute renal failure defined as abrupt or rapid decline in renal filtration function is often transient and usually completely reversible in APSGN. Acute renal failure was present in 56 (76%) in one study and dialysis required in 14, but this was an uncommon finding in our study group.[30,31]

CONCLUSION

APSGN more commonly affects males and is a cause of considerable morbidity. Sore throat as the antecedent infection affects mostly older children as compared to skin infection which tends to precipitate the immune response in the younger age group.

RECOMMENDATIONS

APSGN is a cause for considerable morbidity and suspicion should be kept high in children above 6 years of age till 18 years especially in cases with unusual presentations such as status epilepticus. Blood pressure needs close monitoring to off-set hypertensive emergency and cardiac and neurological complications. Atypical manifestations should be watched for.

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ORIGINAL ARTICLE

ASSOCIATION BETWEEN IRON STATUS AND GESTATIONAL DIABETES MELLITUS

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Background: Early identification of risk factors for gestational diabetes may result in its prevention and better management. In this study, we aimed to compare serum iron, total iron binding capacity and transferring saturation in pregnant women with and without gestational diabetes. **Methods:** A comparative cross sectional analytical study was conducted at Army Medical College and Centre for Research in Experimental and Analytical Medicine (CREAM) in association with Pak Emirates Military Hospital. Thirty healthy pregnant women and thirty patients of gestational diabetes mellitus matched for age, gestational age and body mass index (BMI) were recruited for the study. The diagnosis of gestational diabetes was made on the basis of abnormal glucose tolerance test at twenty four weeks of gestation. Serum iron and total iron binding capacity of all subjects were measured using colorimetric assay and transferrin saturation was calculated. Analysis of data was carried out using SPSS-22. Mean values were calculated for all variables and compared by two sample *t*-test. Pearson correlation was determined to find association between different quantitative variables in two groups and $p \leq 0.05$ was considered significant. **Results:** Serum iron was significantly higher ($p=0.007$), TIBC significantly lower ($p=0.004$) and transferrin saturation significantly higher ($p<0.001$) in gestational diabetes group as compared to control group. **Conclusion:** Markers of iron status including serum iron and transferrin saturation were higher in patients of gestational diabetes and may pose a risk of developing gestational diabetes mellitus.

Keywords: Iron, Total iron binding capacity, Transferrin saturation, Gestational diabetes mellitus

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INTRODUCTION

Gestational diabetes mellitus (GDM) is the most prevalent disease of pregnancy that leads to many complications in both mother and offspring. It is the diabetes that is first diagnosed during second or third trimester of pregnancy and is not clearly overt diabetes.[1] Though it is the most common metabolic disorder of pregnancy, its prevalence varies considerably worldwide depending upon the population as well as the diagnostic criteria used. It has a high prevalence of approximately 14% in Southeast Asia.[2] Though exact data regarding prevalence of GDM in Pakistan is not available, some recent data suggests a prevalence varying from 8 to 26% across various cities of Pakistan.[3] GDM not only poses risk of type 2 diabetes in mother but can also cause life threatening complications in offspring including foetal macrosomia, respiratory distress as well as increased risk of developing diabetes in future.[4] Early diagnosis and management of the condition may result in better outcome for both mother and foetus.

Although exact aetiology of the disease is not known, many factors are considered to promote development of gestational diabetes. Many hormones during pregnancy like oestrogen, progesterone as well as human placental lactogen cause a switch in the metabolism resulting in increased fat utilization and generation of free fatty acids.[5] The imbalance may result in insulin resistance leading to hyperglycaemia and eventually gestational diabetes. The compensation for this developing insulin resistance is made by increased β cell secretion. However, insufficient release of insulin in the background of insulin resistance results in gestational diabetes.[6] Inflammation as well as oxidative stress are considered to be an important factor in pathogenesis of gestational diabetes.[7]

Iron is regarded as a transition metal that has powerful pro oxidant properties. Raised levels of iron may result in oxidative stress that is the basis of many pathologies including diabetes.[8] The oxidative stress not only causes damage to β cells resulting in decreased insulin secretion but is also associated with decreased insulin sensitivity. Moreover, the reactive oxygen species also cause an increase in hepatic glucose output in addition to decreasing uptake of glucose in peripheral tissues.[9] Studies have shown that iron can affect insulin metabolism in the absence of significant iron overload and excess body iron plays a role in impaired glucose tolerance in type-2 diabetes as well as gestational diabetes. However, data is inconsistent regarding relationship between iron status and development of gestational diabetes.[10]

Though additional iron is required for foetus and iron demands are usually increased during

pregnancy, iron loss is also reduced during pregnancy due to absence of menstruation. At the same time, iron supplementation is prescribed too many pregnant females. Usually 30–60 mg supplemental iron is recommended during pregnancy.[11] However, this supplemental iron along with the usual dietary iron intake provides with approximately 16 mg of absorbed iron per day which is much higher than the required amount.[12] In many countries, including Pakistan, there are no proper guidelines regarding iron supplementation and iron supplementation without proper monitoring is a common practice. It is seen that raised levels of iron biomarkers even within the normal range may be associated with development of gestational diabetes especially in those who have iron supplementation in the early pregnancy without its deficiency.[7]

Data regarding iron biomarkers in gestational diabetes patients in Pakistani population is lacking. We aimed to determine serum iron, TIBC and transferrin saturation in patients of gestational diabetes as well as healthy pregnant females and compared the same between two groups to find any association between these and gestational diabetes.

METHODOLOGY

This cross-sectional study was carried out at Physiology Department, Army Medical College and Centre for Research in Experimental and Analytical Medicine (CREAM) in collaboration with Pak Emirates Military Hospital. The study was conducted after the formal approval from Ethical Review Committee of the institute. Using non-probability purposive sampling technique, 30 patients of gestational diabetes mellitus and 30 healthy pregnant women at 24 weeks onwards gestation were recruited. WHO sample size calculator[13] was used to estimate sample size. Considering 3.5% estimated prevalence of GDM in Pakistan and with 95% confidence level along with 5% margin of error, a sample size of 54 was calculated. The subjects in two groups were matched for age, gestational age and BMI. Oral glucose tolerance test was carried out and diagnosis of gestational diabetes was made according to the criteria established by International Association for Diabetes and Pregnancy Study Group (IADPSG).[14] The demographic data and history were recorded on the proforma. Relevant clinical examination was carried out. Participants fulfilling inclusion criteria were selected for study and those with anaemia, haemoglobinopathies, hormonal disorders including type 1 and 2 diabetes and past history of gestational diabetes were excluded.

For biochemical tests, 5 ml of blood was collected through peripheral venipuncture. Glucose Tolerance Test with 75 gram glucose was performed after an overnight fast of at least 8 hours. For estimation of plasma glucose, blood was collected in collection tubes containing sodium fluoride and potassium oxalate. Analysis of plasma glucose was performed using Roche Cobas C 501 analyser. For iron biomarkers, blood was collected in serum separator tubes and allowed to clot, after which it was centrifuged and serum was collected within an hour of sample collection. Serum iron and TIBC were determined by colorimetric method using Roche Cobas C 501 system. Transferrin saturation was calculated by dividing serum iron with TIBC and was expressed as percentage by multiplying the result with 100. Data were analysed through SPSS-22. Two sample *t*-test was used for comparing mean values of all biochemical parameters between two groups. To find association between plasma glucose and serum iron as well as transferrin saturation, Pearson's coefficient was determined, and $p \leq 0.05$ was regarded as significant.

RESULTS

Serum iron levels were significantly higher in gestational diabetes patients compared with control group (p=0.007). Total iron binding capacity was lower in GDM patients compared to controls (p=0.004). Transferrin saturation was higher in gestational diabetes group compared to control group (p<0.001).

Table-1: Comparison of mean values of fasting blood glucose, serum iron, TIBC and transferrin saturation between two groups

Test parameters	Control group	GDM group	*p*
Fasting plasma glucose (mmol/L)	4.52±0.47	5.98±1.04	<0.001*
Serum iron (µg/dL)	52.21±8.28	65±25.17	0.007*
TIBC (µg/dL)	439.3±168.32	325.39±146.24	0.004*
Transferrin saturation (%)	13.43±4.85	22.86±10.9	<0.001*

*Significant $p \leq 0.05$

A positive correlation of serum iron and transferrin saturation was found with fasting plasma sugar, 1-hour and 2-hour postprandial plasma glucose (Table-2). All these correlations were statistically significant except that between serum iron and 2 hour plasma glucose.

Table-2: Correlation of serum iron and transferrin saturation with plasma glucose

Parameters correlated	*r*	*p*
Serum iron		
Fasting plasma glucose	0.306	0.01*
1-hour plasma glucose	0.281	0.02*
2-hour plasma glucose	0.158	0.19
Transferrin saturation		
Fasting plasma glucose	0.385	0.001*
1-hour plasma glucose	0.401	0.001*
2-hour plasma glucose	0.372	0.002*

*Significant $p \leq 0.05$

DISCUSSION

We compared iron status of normal pregnant women with patients of gestational diabetes mellitus as reflected by serum iron, TIBC and transferrin

saturation. We found a significant high serum iron, a low TIBC and high transferrin saturation in gestational diabetes group as compared to control group. Also, these parameters correlated positively with fasting as well as 2-hour plasma glucose levels.

Iron being transition element is involved in generation of oxidative stress which may play a role in development of gestational diabetes.[15] Considering the increased demand of iron by foetus during pregnancy, iron supplementation is a common practice and is exercised in many developing countries without prior confirmation of its deficiency. Though WHO recommends 30–60 mg elemental iron as supplementation in pregnancy[16], there are no proper guidelines regarding ion supplementation in many developing countries[17]. In a few cases, iron supplementation is self-prescribed both before and after pregnancy. Iron supplementation in already iron replete women may result in more harm than benefit as is shown in few earlier studies.[18–20]

We included thirty healthy pregnant women at 24 weeks onwards gestation and thirty women with gestational diabetes mellitus. A few other similar studies also included comparative number of subjects in their study.[21] We diagnosed gestational diabetes by conducting oral glucose tolerance test at 24 weeks of gestation and applied IADPSG criteria for diagnosis as recommended by WHO.[14]

Significant higher serum iron levels was observed in GDM group than controls. A study conducted in China by Lao *et al* revealed significant high levels of iron in GDM patients as compared to healthy pregnant women. They measured these levels between 28 to 30 weeks of gestation.[22] The mean serum iron in their study is comparable to that found in our study, though they studied a larger group of subjects and included 97 GDM patients and 194 healthy subjects. Another recent study conducted on 150 subjects showed similar results with higher serum iron in GDM patients in all age groups.[23]

Afkhami-Ardekani conducted a study recruiting subjects during 24 to 28 weeks of gestation and found similar results.[21] The study was conducted in Iran where similar socioeconomic and regional factors exist. Serum iron in their study was significantly higher in GDM group. Another study conducted in Iran by Behboudi-Gandevani showed similar results with relatively higher serum iron levels in all subjects.[24] The relatively higher values of serum iron may be because they measured them at earlier gestation of 14–20 weeks and then performed OGTT at 24–28 weeks of gestation.

Derbent *et al* measured serum iron in 30 GDM patients and 72 healthy controls around same time of gestation and discovered statistically significant difference between the two groups.[25]

A few earlier studies compared TIBC in GDM patients and healthy controls and obtained diverse results. Lao *et al* found statistically significant lower TIBC in GDM patients in addition to higher serum iron.[22] Similar results were found in some other studies.[21,25]

We also calculated transferrin saturation and found it to be significantly higher in GDM patients than in healthy controls. Our finding is consistent with earlier work.[21]

CONCLUSION

Serum iron and transferrin saturation were significantly higher in gestational diabetes patients as compared to normal pregnant women. Iron excess may pose a risk for development of gestational diabetes.

LIMITATIONS OF THE STUDY

The limitations of the study were its cross-sectional design in addition to a relatively smaller sample size considering the vast variation in diet, life style and genetics of population. Further prospective studies should be conducted to elaborate the role played by iron in occurrence of gestational diabetes mellitus. There is a need to review the practice of routine iron supplementation during pregnancy without iron deficiency.

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ORIGINAL ARTICLE

EFFECTS OF CLINICAL INTERVENTIONS ON NURSING CARE PRACTICES REGARDING INCIDENCE OF PRIMARY POST-PARTUM HAEMORRHAGE

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Background: Postpartum haemorrhage (PPH) is a common obstetric haemorrhage. That is one of the leading causes for maternal mortality. This study aims to assess the effect of clinical interventions on nursing care practices regarding primary postpartum haemorrhage. **Methods:** This quasi-experimental study was conducted in Gynaecology Department of Allied Hospital, Faisalabad. Eighty-five license holder nurses who work in morning, and evening shifts, aged 25–50 were included. Nurses who had PPH training and/or teach obstetrics, gynaecology and midwifery students, were excluded. Purposive sampling technique was used. A 16-items adopted validated checklist was used to assess the practices of nurses pre- and post-educational training about primary PPH. **Results:** Demographic and skill related variables were checked for frequency and percentages. All of nurses (n=85) had poor practices before clinical interventions. In post-intervention assessment, results revealed that 11 (12.9%) nurses had good and 74 (87.3%) showed excellent practices, and no one had poor practices. **Conclusion:** Educational trainings had prominent effect in improving skill performances and practices of maternity nurses regarding the management of labour to prevent primary post-partum haemorrhage.

Keywords: Postpartum haemorrhage, PPH, Spontaneous vaginal delivery, SVD, nurses, practice

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INTRODUCTION

A blood loss of 500 ml within the 24 hours of childbirth is termed as postpartum haemorrhage (PPH). It is the primary cause of almost one quarter of maternal deaths around the globe and central cause of maternal mortality in developing countries.[1] One of the most unanticipated consequences of childbirth is postpartum maternal morbidity. It is estimated by the World Health Organization (WHO), United Nations Population Fund, United Nations International Children Fund, United Nations, (UN) and World Bank Group that around 303,000 maternal deaths happened in 2015 in developing countries accounting for 99% of the global maternal deaths.[2] Abnormal uterine atony, retained products of placenta, laceration of genital area can be categorized as PPH. After delivery, lack of fundal message and delay administration of Pitocin can lead to uterine atony and haemorrhage.[3]

One of the preventable cause of maternal mortality and morbidity is Post-partum haemorrhage. In low income countries high prevalence rate indicate the need for evidence based practice among healthcare providers to prevent and manage PPH. Obstetric nurses should carried appropriate knowledge and skill to meet the patients demand.[4] Improper implementation of labour room guidelines by nursing professionals propose that there is vast difference between recommendations and actual practice. Proper implementation of guidelines for postpartum haemorrhage prevention and management can significantly reduce incidence rate of PPH.[5]

Appropriate duty performance by nurses can significantly reduce the incidence of PPH, however, at project site labour room negligence is noted in nursing responsibilities. Routine examination of perineal area to find out and laceration is important because sometimes sphincter lacerations go unidentified by obstetrician and lead to postpartum haemorrhage.[6] Blood pressure monitoring, heart rate, position and fundal tone, per vaginal blood loss every 15 minutes interval should be included in nursing observation. A typical method for assessing blood loss is by weighing sanitary pads hourly and assess the lochia for blood clotting. These duties are performed by labour room nurses but there performance is not sufficient in term of preventing postpartum haemorrhage.[7]

Blood loss after delivery is continuously miscalculated by labour and delivery room nurses, they do not perform fundal massage appropriately, which could be the reason to delay in providing care during PPH.[8] The goal of this study is to assess the effect of clinical interventions on nursing care practices regarding primary postpartum haemorrhage prevention.

METHODOLOGY

This quasi-experimental study investigated the nursing care practices of 85 registered nurses from Gynaecology Department of a teaching hospital in Faisalabad Pakistan from September to February 2022. Seventy one License holder Nurses who work in morning, and evening shifts, aged 25–50 years were included. Nurses who had PPH training and those who were teaching Obstetrics, Gynae, and Midwifery students, were

excluded. Purposive sampling technique was used. Following formula was used to calculate the sample size, and 194 was calculated with 95% confidence interval, 0.7% margin of error, and expected percentage of improved practice score as 55.4%.[9]

$$n=\frac{z^2_{1-\alpha/2}\ P(1-P)}{d^2}$$ 1 _ α=95 d= 0.07

n=194 P=0.554[9]

After adjustment of the calculated sample size, i.e., 194 due to small population size (110) the sample size was taken as 71. After adding up 20% drop out rate the sample size was 85.

$n=\frac{n0}{(1+(\frac{n0}{N}))}$, where n_0=194, N=10, n=71

Clinical assessor observed the participants in pre-assessment, for care practice at their job place by using checklist. Eight weeks of educational training regarding Primary Post-Partum Haemorrhage was delivered. Validated educational training was delivered by the relevant doctors of the related field. In 16 weeks clinical intervention nurses were taught for skill competence by the simulation, videos, notes and live performance on patients. Intervention was given into two groups for 2 hours in a week. For improving practices, 4 weeks were given. Then changes in their care practice, participants were reassessed.

Sixteen items validated Practice Checklist was used. Accurately performed practices were ticked under the column of 'Yes' and the score was marked as 1, missed or wrong one step as '0' under the category of 'No'. Categories of skill competency was made as Poor practices if score was ≤50% (≤8 points), Good practices with >50–75% (9–12 Points), and Excellent practices as >75% (>12 points).[9]

For ethical concerns, institutional review board approval was taken from the University of Lahore, Lahore. From all participants signed consent was taken and information was kept confidential.

SPSS-20 was used for data entry and analysis. For professional and demographic variables, frequency and percentage were checked. Pre and post educational training, data regarding competence of skill was taken two times, from one group. Pre data was coded as 1 and post data as 2, and $p \leq 0.05$ was considered significant.

RESULTS

Total 85 nurses were chosen from a teaching hospital of Faisalabad. Ages of 30 (35.3%) participants were 25–30 years, 34 (40.0%) was between 31–35 years, 15 (17.6%) was between 36–40 years of age and 6 (7.1%) were between 40–45 years, all were females. Education status revealed that 4 (4.7%) nurses were diploma holder, 30 (35.3%) research participants had Bachelor degree in nursing, and 51 (60%) research participants had Post Rn nursing degree. Job experience showed that 17 (20.0%) nurses had their experience less than 1 year, 20 (23.5%) research participants had 1–2 years, 28 (32.9%) nurses job experience was between 3–4 years and 20 (23.5%) study subjects had working experience ≥5 years.

Table-1 shows the frequency and percentage of correctly performed nursing care skills during spontaneous vaginal delivery, pre- and post-clinical intervention. Table-2 depicts the comparison of nursing care categories (poor, good and excellent practices) pre and post educational training to nurses working in gynaecology department. In Table-3 mean of post clinical intervention, nursing care competency score was higher than the mean of pre intervention skill competency scores.

Table-1: Pre and Post Clinical intervention, care practices of nurses about Primary PPH [n=85, n(%)]

Items of Checklist	Before Intervention Correct Practices	After Intervention Correct Practices
Checked presence of another foetus	30 (35.3)	73 (85.9)
Accurate timing for administration of uterotonic drug	27 (31.7)	76 (89.4)
Uterotonic drugs types given, i.e., Oxytocin or Ergometrine	71 (83.5)	69 (81.2)
Correct dose of uterotonic drugs given	22 (25.7)	73 (85.9)
Uterotonic drugs, correct mode of administration	38 (44.7)	69 (81.2)
Correct timing of cord clamping	34 (40.0)	72 (84.7)
Waited 2–3 min for uterine contraction, to apply CCT	29 (34.1)	67 (78.8)
To apply cord control traction, wait for gush of blood	14 (16.5)	74 (87.1)
Placenta delivered before uterotonics administration	24 (28.2)	74 (87.1)
CCT performed as protocol	41 (49.4)	64 (75.3)
Placenta was supported by both hands	35 (41.2)	68 (80.0)
With lateral movement, membrane extracted gently	30 (35.3)	71 (83.5)
Uterine massage immediately after delivery of placenta	35 (41.2)	79 (92.9)
Placenta assessed for completeness	29 (34.1)	69 (81.2)
Uterine relaxation ensured	35 (41.2)	77 (90.6)
Inform and demonstrate the mother massage uterus	29 (34.1)	71 (83.5)

Table-2: Comparison of pre and post clinical intervention, care practices of nurses regarding primary PPH [n=85, n (%)]

Categories of nurses' practices	Scores	Pre-intervention scores	Post-intervention scores
Poor	≤8 points (≤50%)	85 (100%)	0 (0%)
Good	9–12 points (51–75%)	0 (0%)	11 (12.9%)
Excellent	>12 points (≤75%)	0 (0%)	74 (87.3%)

Table-3: Paired *t*-test between before and after intervention clinical practices of nurses (n=85)

Score	Mean±SD	Mean difference	Paired *t*-test	*p*
Pre intervention	6.282±1.12	-7.4	-41.44	0.00
Post intervention	13.494±0.995			

DISCUSSION

Skilful nursing management during and after labour is among lifesaving interventions. Educational interventions have been evidenced to decrease the stress and anxiety in emergency scenarios and enhance the nurses confidence to manage patients during labour. Educational trainings is a helpful way to improve practices of maternity nurses. It may enhance their skills and self-confidence.[10]

Results of present study proved that, before educational intervention all maternity nurses had poor practices during the management of labour to prevent mothers from primary postpartum haemorrhage. After educational training their practices improved to good and excellent. No one maternity nurse had poor practices. These findings are aligned with Shakur *et al*[11] who found that pre simulation training, nurses had unsatisfactory skills in management of labour, while after intervention there was significant improvement. Another study showed same results that simulation based interventions improved nurses' practices during labour to prevent from postpartum haemorrhage.[12]

A few studies contradict the findings of current study, their results showed that there was no effect of educational interventions on maternity nurses knowledge and practices regarding management of third stage of labour to prevent primary postpartum haemorrhage.[13,14]

CONCLUSION

Clinical educational trainings had prominent effect in improving skill performances and practices of maternity nurses regarding the management of labour to prevent from primary postpartum haemorrhage.

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AY: Assisted in designing and gave critical inputs in manuscript
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ORIGINAL ARTICLE

RISK FACTORS AND PARENTS' ATTITUDE FOR CHILDREN PRESENTING WITH ASTHMA AT A TERTIARY CARE HOSPITAL

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Background: Asthma is the most commonly prevalent disease in children. There is increasing prevalence and incidence of asthma in children in last few decades. Not only risk factors but also parent's attitude towards therapy matters the most in management. Objective was to assess for the risk factors and parents' attitude for children presenting with asthma. **Methods:** This cross sectional study was done at Ayub Teaching Hospital, Abbottabad. History was taken from parents about risk factors and associated myths. Duration of asthma, visits per year to clinic/hospital, tobacco smoke exposure, pets, fur toys, wood/coal burning, carpets in rooms, perfumes/powder use was documented. Parents' attitude towards use of spacer and inhaler was documented with specific questionnaire. Data was analysed using SPSS-20 and $p<0.05$ was taken as significant. **Results:** There were 136 patients, 93 (68.4%) males and 43 (31.6%) females. Mean age was 7.3±3.13 years. Most (80.1%) patients were <10 years old. Patient visits per year ranged from 2 to 15 visits with mean of 5.97±3.02 visits per year. Intermittent asthma was seen in 8.8% and persistent asthma was seen in 66.9% patients. The most common risk factor was use of perfume in 48.5% and family history in 65.4% patients. Parents' misconception towards inhaler use was noted in 38.2% patients. Younger children are at more risk of having asthma symptoms with exposure to wood/coal smoke and perfume powder exposure ($p=0.04$). **Conclusion:** Family history of asthma along with perfume/powder exposure and wood/coal smoke exposure are major risk factors in children. Parents' misconception is also a hurdle to timely management.

Keywords: Asthma, risk factors, myths, childhood

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INTRODUCTION

Childhood asthma is one of the common chronic disease not only affecting children only but also parents and families.[1] There is increasing prevalence and incidence of asthma in children in last few decades.[2] In Pakistan though exact prevalence of asthma is not known yet it is reported as 10.1% in children 13–14 years old.[3] Non adherence to doctor advice and exposure to environmental risk factors are the factors leading to exacerbation of asthma.[4] Also due to poor parental knowledge and myths associated with use of inhaler/spacer for asthma, there is inappropriate management of children with asthma.[5]

In our part of world there are many myths associated with use of inhaler in children as it will damage the lungs or child will remain dependent on inhaler. Also there are many risk factors to which patient is exposed but caregivers do not know and this leads to exacerbation and poor control of symptoms. This study is one step in recognition of the risk factors and parents attitude. This will help the paediatricians to find out the lacking data and give better management plan to patients. Objective of this study was to assess for the risk factors and parent's attitude in children presenting with asthma.

METHODOLOGY

This study was conducted in OPD of Paediatric B Ward, Ayub Teaching Hospital, Abbottabad form January 2018 to December 2019 over two years. After approval from Institutional Review Board, consent was taken from parents and then patients were included in the study. Children from 2 to 15 years of either sex were included. Children who were diagnosed case of asthma were included. Also the children with history of cough and wheeze for at least four weeks with exclusion of other pathologies were taken as case with asthma and complete the criteria of Global Initiative for Asthma (GINA) guidelines 2015 were included. Children known case of chronic lung disease including cystic fibrosis, primary ciliary dyskinesia, tuberculosis, immunodeficiency, congenital heart diseases, and chest X-ray suggestive of infection were excluded. Patient age, weight, sex, duration of asthma, visits per year to clinic/hospital, tobacco smoke exposure, pets (including pigeon, parrot, dog, cat at home), toys with fur, wood and coal burning, carpets in rooms, use of perfumes or powder were documented on specific proforma. Also parents attitude towards use of spacer and inhaler was documented with specific questionnaire that if they consider it to cause dependency or not and will lead to

permanent lung damage. The responses of parents were documented. Patient symptoms were classified according to guidelines and patient was classified as case of asthma as intermittent and persistent. Then persistent asthma was classified as mild, moderate and severe type on basis of symptoms. Data was analysed using SPSS-20. Chi-square test was applied and results were taken significant if $p<0.05$.

RESULTS

There were total of 136 patients included in this study. Out of 136 patients, 93 (68.4%) were males and 43 (31.6%) were females. Age of patients ranged from 2.5 year to 15 years with mean age of 7.3±3.13 years. Patient's age was categorized as 2 to 5.0 years, 5.1 years to 10.0 years and above 10.1 years. Age category and asthma percentage given in Table-1. There is significant association between age category and woo/coal smoke exposure with $p=0.040$. The younger children are at more risk of having asthma symptoms with exposure to wood/coal smoke exposure. There is also significant association of perfume/powder exposure to risk of asthma in younger children with $p=0.040$. Weight of patients ranged from 10 to 50 Kg with mean weight of 20.765±7.10 Kg. Duration of patient symptoms ranged from 3 months to 10 years with mean duration of 29.67±27.38 months. Patient visits per year ranged from minimum of 2 visits to maximum of 15 visits with mean visits per year of 5.97±3.02.

Out of 136 patients, 12 (8.8%) patients were with intermittent asthma, 91 (66.9%) patients were having mild persistent asthma, 29 (21.3%) patients were with moderate persistent asthma and 4 (2.9%) patients were with severe persistent asthma. The most common risk factor identified in history was use of perfume or powder by children, as 66 (48.5%) patients were using perfume/powder. The second most risk factor followed by use of perfume/powder was carpets at home as parents do not consider them to be predisposing factor for asthma in children. Carpets were present in 59 (43.4%) patients home. The third most important risk factor identified in this study was tobacco smoke exposure which was present in 51 (37.5%) patients. Other risk factors identified were pets (pigeon/parrot/ dog/cat) in 36 (26.5%) cases, fur toys for children in 36 (26.5%) patients, wood/coal fire smoke exposure in 37 (27.2%) patients. Family history of asthma was present in 89 (65.4%) patients while in 47 (34.6%) patients there was no family history of asthma.

Parent's misconception towards inhaler use was noted in 52 (38.2%) patients. Parents of these patients either consider inhaler harmful for the lungs or of opinion that child will be dependent on inhaler in future which may also be in adulthood as marked on the specific questionnaire. Though there were only four patients with severe persistent asthma, yet 75% of parents of these patients had myth about inhaler use (Table-2). There is significant association between wood/coal smoke exposure and parents misconception about inhaler use with $p=0.015$.

Table-1: Age category

Age group	Frequency	Percent
2–5 years	49	36.0
5.1–10.0 years	60	44.1
10.0 years and above	27	19.9
Total	136	100.0

Table-2: Parent's misconception toward inhaler use *vs* asthma classification —Cross tabulation

Parent's misconception about inhaler use	Asthma classification				Total
	Mild persistent	Moderate persistent	Severe persistent	Intermittent	
Yes	38	8	3	3	52
No	53	21	1	9	84
Total	91	29	4	12	136

DISCUSSION

Asthma prevalence in one city of northern part of Pakistan is estimated around 31.5%.[6] Though no age group is immune from developing asthma yet it is more prominent in children as parents and family involved in care of child noticed the symptoms.[7] In management of childhood asthma control of risk factors associated with exacerbation is the central pillar. Parents understanding of disease and its treatment are also one of the key aspects in management of children with asthma. This study was done to assess for the risk factors and parent's attitude in children presenting with asthma.

In one review article, Hui RWH studied the reasons of not using steroid based inhalers and prevalence about the myths with inhaler use in children. Hui RWH estimated the prevalence about not using steroid based inhaler in 19–67% of patients in different populations and main reasons for not using these inhaler were related adverse affects including weight gain, growth suppression, weakening of bones, psychiatric disturbance and addiction to inhaler.[8] While in our study the prevalence of not using the inhaler was 38.2% and main concerns of parents were addiction dependence on inhaler and lung damage.

Majeed R *et al*[9] studied the risk factors in children with asthma. Their study included children aged from 12 months to 8 years while in our study age ranged from 2 years to 15 years in their study 60% of patients were male, in comparison in our study 68.4% were males. There is history of asthma in family and 38.5% patients had exposure to smoking. In our study 65.4% patients had family history of asthma while 37.5% patients had exposure to tobacco smoke which is almost equal to Majeed R *et al* study findings. In one study done by Afzal M *et al*[10] for risk factors in children and found that tobacco smoke exposure and history of asthma in family were the two important

factors. In our study these two factors were also very significant.

Karunasekera *et al*[11] studied environmental and genetic factors in children with asthma. Childhood asthma risk in increased in children with family history of asthma either in father or mother. One of the main environmental factor recognized was firewood smoke. In our study fire wood exposure was in 27.2% patients and family history of asthma was in 65.4% patients. Khan IM *et al*[12] did one study about the impact of socioeconomic status of parents on asthma control in children. It was concluded in their study that children of parents with low income have poor control of asthma as compare to children whose parents have high income. In our study we did included the income of parents but included the exposure to firewood smoke. As families in whom income is low or from rural area tend to use fire wood for cooking and in 27.2% of patients there was history of firewood or coal smoke exposure. Kamran A *et al*[13] in their study concluded that apart from living area without sunlight and room without window, the major risk factors in childhood asthma were tobacco smoke exposure at home and family history of asthma. In our study family history of asthma was present in 65.4% patients and tobacco smoke exposure was present in 37.5% patients.

Basharat S *et al*[14] study included children with persistent asthma for drug adherence and control of symptoms. In our study the persistent asthma was in 91.2% patients. In Basharat S *et al*[14] study 65.17% were male and 34.83% were female while in our study 68.4% were male and 31.6% were female. The childhood asthma is more prevalent in males as compare to females. Simba J *et al*[15] did one study in one referral center of Kenya about the care takers perceptions and knowledge about childhood asthma. In their study it was found out that care takers prefer syrups to inhaler in 70.7% of cases. Though we did not take opinion regarding syrup use but 38.2% patient's parents were reluctant in use of inhaler due to myth that it leads to dependency or there is lung damage with use of inhaler.

Al-Anazi A *et al*[16] assess the level of parent's awareness in their study about childhood asthma. They used Likert scale questions for assessing parent's awareness about childhood asthma. In one of the category they checked for the myths and beliefs about asthmain children. The questions about inhaler dependence and not good for long use scored 3.39±1.32 and 2.69±1.32 respectively. In our study the parents of 38.2% children had myths leading to dependence and lung damage in 38.2% of children. Noureddin AA *et al*[17] did their study in asthma clinics in Sudan. They found out that 21% of mothers of children with asthma consider inhaler use not acceptable at all. In our study the myths related with use of inhaler leading to not use of inhalers was in 38.2% children.

Ankermann T *et al*[18] in their review article concluded that there is vaccination in routine does not predispose the children to allergies including asthma. Labuschagne IL *et al*[19] in their review study concluded that diet with vitamins including vitamin C, D, E and polyunsaturated fats have protective role in children with asthma.

There were limitations in this study. We only considered specific factors. One of important environment risk factor is moulds exposure which was not considered. Another factor was parent's adherence to preventive medications which was also not included. We also did not consider the nutritional history and nutritional status of children.

CONCLUSION

Family history of asthma along with perfume/powder exposure, wood/coal smoke exposure, carpets at home and tobacco smoke exposure at home are the major risk factors for childhood asthma. Parents' misconception towards use of inhaler is one of major hurdle in asthma symptom control and prevention.

RECOMMENDATIONS

Parents' education and awareness about the use of inhaler for preventive and controller medicines is needed both at community and hospital level along with the focus on parents' attitude and misconception.

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ORIGINAL ARTICLE

IMPACT OF SARS-COV-2 ON FRONTLINE HEALTHCARE WORKERS IN AZAD KASHMIR: A MULTICENTRE SURVEY

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Background: Healthcare workers (HCWs) are especially prone to contracting SARS-CoV-2 infection due to their work requirements. This study was conducted to analyze the frequency of SARS-CoV-2 infection among frontline healthcare workers and various predictive factors of this infection in healthcare workers. **Methods:** This was a descriptive cross-sectional study. Data was collected at Obs/Gyn Department, Sheikh Khalifa Bin Zayed Al-Nahyan (SKBZ)/Combined Military Hospital Muzaffarabad, from Main Covid-19 Pandemic Data Collection and Information Centre, Muzaffarabad, from 15th April to 14th October 2021. Screening was offered to healthcare professionals and other supporting staff of all hospitals dealing with SARS-CoV-2 infection. Information was recorded on a structured questionnaire. Categorical variable were explained by frequency. Fischer exact test and χ^2 test were used, and $p<0.05$ were considered significant. **Results:** There are 4,776 healthcare workers in AJK. A total of 2,219 individuals were COVID positive, out of them 118 were healthcare workers. Males were 53% and females were 47%; 77% of cases were aged 25–50 years. Physicians were 51.7% outnumbering nurses and allied staff. The cumulative incidence was 2.4%. Infection rate among frontline HCWs was 1%; it was 1.6 among HCWs in other departments, and 0.5% in HCWs with no patient contact. The rate of co-morbidities in affected HCWs was 33.05%. **Conclusion:** The risk of disease was high among frontline healthcare workers as compared to general population. The severity of infection was more in patients with co-morbidities. Among HCWs, the highest number of patients with COVID-19 were physicians.

Keywords: SARS-CoV-2, Impact, frontline healthcare worker, COVID-19, Coronavirus

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INTRODUCTION

Since the outbreak of SARS-COV-2 a novel coronavirus healthcare worker (HCWs) are over burdened by increased duty hours after it has been declared as a pandemic by the World Health Organization.[1] This pandemic put healthcare system under tremendous pressure and challenged its ultimate capacity to deal with fast past infectious virus. Healthcare professional took responsibility and shouldered policy makers with utmost efforts in line of their duty.

In the study presented herein, we have tried to underscore some of the imperatives and challenges currently being faced by frontline health workers in Azad Jammu and Kashmir State and propose certain recommendations to reduce the impediment being imposed on them in order to ensure the provision of rapid and efficient health care services.

Earnest service and unconditional approach has always been considered as fundamental ethic for all health workers (HCWs), especially in time of peril as the present day. The last 15 months have been unusual for every individual across the globe as it has set the new norms for worlds living with COVID-19. SARS-CoV-2 pandemic has not only implicated widespread morbidity and mortality but it has created fear and burden among HCWs and put everything on emergency mode. Reports across the world reveal that COVID-19 has resulted risk for anxiety, depression, insomnia, and post-traumatic stress disorder among front line health care workers. It has also been shown that severe burnout syndrome affects as many as 33% of critical care nurses and up to 45% of critical care physicians that reflects the magnitude this virus has taken a toll on the HCWs.[2]

Back in 2003 outbreak of Severe Acute Respiratory Distress Syndrome or SARS was reported for the first time in Hanoi, Vietnam. Outbreak of COVID-19 seems a repeat history, as reflected by the data presented by Chinese National Health Commission, with statistical data revealed that more than 3,300 HCWs got infection in first of week of March with at least 46 deaths till mid of march. Similarly, in Italy 20% of their HCWs were infected, with significant number were reported losing their lives to the virus.[3,4] Those who survived COVID-19 infection developed certain degree of immunity against SARS-CoV-2 as shown by the antibody titres done after six month of recovery.[5,6] Only effective vaccination against COVID-19 can

prevent it in near future. As virus transmission percentage is not exactly known so it is difficult to assess the seropositivity of population but it is estimated to range between 60% and 80%.[7,8]

Until this compilation in Pakistan there have been 332,993 cases and 6,806 deaths (as 29th October, 2020, 1700 hours) reported officially by Ministry of Health. Meanwhile in Azad Jammu and Kashmir (AJK), there have been 4,082 cases and 89 deaths (as of 29th October, 2020). Now another wave of SARS-CoV-2 has set in and frontline healthcare workforce is highly exposed to SARS-CoV-2 infection again. Screening of HCWs with COVID-19 RT-PCR is of pivotal importance for surveillance of the pandemic and to predict the likely course herd immunity. Surveillance of the proportion of COVID-19 positive health care workers is an important indicator of spread of SARS-CoV-2.

We aimed to see the frequency of COVID-19 infection among frontline healthcare workers in AJK healthcare system and to analyze various predictive factors of this infection.

METHODOLOGY

A total of 118 healthcare workers, who were found SARS-CoV-2 positive were included in the study from April to October 2020. Information regarding epidemiological factors, exposure, symptoms and others relevant clinical information was taken on a structured questionnaire. Frontline or first line healthcare workers were defined as HCW directly involved in care of confirmed or suspected COVID-19 cases, or stationed at emergency. Non-first line HCWs were those HCWs who were involved in general patients other than COVID-19. Categorical variable were explained by frequency. Chi-square test and Fischer exact test were used as per type of variable. SPSS-24 was used for data analysis. Two sided $p<0.05$ was considered significant.

RESULTS

There were 4,776 HCWs working actively in AJK. Out of a total number of 2,219 patients diagnosed in AJK during the study period, 118 were healthcare workers, 52% were males and 47% were female, 77% cases were aged 25–50 years, median age was 33 years. Physicians were 51.7% and they outnumbered nurses and allied staff. The cumulative incidence was found to be 2.47%. (Table-1).

COVID-19 infection was found to be 24% among the frontline HCWs, 76% among workers in other clinical departments and those with no direct contact with the patient (59% & 14% respectively). Infection rate was 0.8% among frontline healthcare workers, 1.4% among workers in other clinical departments and 1.6% among workers with no patient contact. (Table-2).

Co-morbid conditions associated with 118 COVID-19 confirmed HCWs are shown in Table-3. The rate of co-morbidities in affected HCWs was (39/118, 33.05%). Common co-morbidities among all COVID-19 positive individuals were diabetes (21, 17.79%), hypertension (8, 6.77%), cardiovascular disease (4, 3.38%), asthma (2, 1.69%). Three (2.54%) of SARS-CoV-2 affected women were pregnant.

Fever was the most common presenting symptom. Majority (102, 84.3%) of affected HCWs had mild course of disease while disease was severe in 12 (9.9%) cases. CPAP was used in 12 patients. Four (3.3%) had critical disease, out of which 2 frontliners died. Fatality rate among HCWs was 1.69%. Infectivity was higher in diabetic and in hypertensive individuals and majority presented with features of common flu. (Table 4).

Seventy-two (61%) HCWs got infection presumably from the Ward, 28 (23.7%) from Emergency Department/Clinic, while 18 (15.2%) were infected in community. Only 3 had travel history to cities with high prevalence of Covid 2 cases. More (72, 61%) second line workers were infected than frontline HCWs in non-COVID wards ($p<0.001$). Twenty-eight HCWs had presumably transmitted infection to their families as well. Comparison of co-morbidities and symptoms between frontline and non-frontline HCWs found no significant difference in both subgroups. Age group 26–50 was the most affected group. (Figure-1).

Table-1: Frontline HCWs according to their category and workplace

	HCWs	HCW with COVID-19	HCW without COVID-19	Estimated cumulative Incidence
Total	4776	118	4658	2.47
Job category				
Physician	1135	61	1074	5.37
Nurses	649	10	639	1.54
Assistants (paramedics)	2992	47	2945	1.57
Department				
Outdoor and wards	3284	100		3.05
Other departments	1492	18		1.21

Table-2: Exposure location and job categories of HCWs with confirmed SARS-CoV2 infection

Parameters	Total	Frontline HCW		% of frontline HCWs
		Yes	No	
Presumed exposure location				
ER/OPD	24	20	4	16
Wards	76	5	71	4
Other	18	5	13	4
Job category				
Physicians	61	30	31	51.6
Nurses	4	4	0	3.3
Allied	6	6	0	5
Transmitted to family/ friends	28	13	15	

Table-3: Co-existing diseases in HCWs with confirmed SARS-CoV2

Clinical Features	Total	Frontline HCW	
		Yes	No
Diabetes mellitus	21	9	12
Hypertension	8	2	6
Cardiovascular Disease	4	0	4
Pregnancy	3	1	2
Asthma	2	1	1
Renal disease	1	0	1

Table-4: Signs and symptoms in confirmed positive HCWs (n=118)

Signs and symptoms	Total	Frontline HCW		
		Yes	No	*p*
Fever	78	34	44	0.002
Cough	45	17	28	0.55
Body aches	68	30	38	0.01
Sore-throat	39	16	29	0.30
Shortness of breath	27	12	19	0.79
Loss of taste	4	3	1	0.112
Loss of smell	10	5	5	0.304
Diarrhoea	7	5	2	0.04
Headache	8	4	4	0.441
Anorexia	3	2	1	0.269
Photophobia	1	1	0	0.339
Nausea	2	2	0	0.113

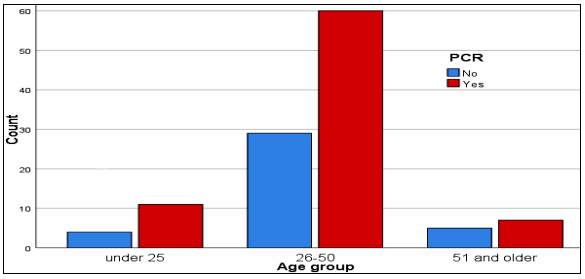

Figure-1: SARS-CoV-2 infection in age groups

DISCUSSION

This study started in Gynaecology Department of SKBZ Hospital Muzaffarabad. Later on, all district and teaching hospitals in Azad Jammu and Kashmir were included in the study. These hospitals are catering patients from all over the State, and HCWs were at higher risk of getting viral infection.

The risk of acquiring the infection from patients is more in HCWs due to their direct and prolonged contact with patients suffering from COVID-19. The HCWs may not notice contracting the disease for a while. When compared SARS and Middle East Respiratory Syndrome with COVID-19, it was noticed that COVID-19 infection has much longer incubation period than the former infections.[9] Longer incubation period results silent propagation of virus to others without showing any symptom.[10] Many COVID-19 patients visiting hospitals had no or very subtle symptoms and some showed atypical symptoms. Presence of such patients in hospital setting poses greater risk for health staff and clinicians over there.[11–13]

The data analysis stratified with age reflected higher involvement of younger (21–30 years) healthcare workers with a successive declining trend with an increase in age. The reason behind this trend was higher proportion of this age group being in direct contact with the patients and for longer period of time. Similar observations were made by Cortis D[14]. However, the severity of the disease in this group was lesser than those with advancing age because of lesser number of workers with co-morbids and better immunity for long working hours with infective patients. More male than female healthcare workers were involved probably due to social limitations of female workers. Global results suggest that men and women are equally affected, except in Pakistan where 72% cases were male, and the disease severity and mortality was more among males.[15]

COVID-19 is associated with a varying degree of disease, its course and common symptoms including fever, cough, sore throat, myalgias and shortness of breath. Diarrhoea remained an uncommon symptom in our study population. This is in agreement with Wei-Jie Juan *et al*[16]. Fever was the most common symptom in our location. Other common symptoms were cough, body aches, sore throat, and shortness of breath. Loss of taste and smell were less prevalent among all confirmed cases of COVID-19.

Among 4,776 healthcare workers 118 (2.47%) were affected with COVID-19, out of which 39 (33.05%) had associated co-morbidity. Among these patients the most common co-morbidity was diabetes followed by hypertension and IHD; pregnancy, asthma and renal disease were least associated. A study conducted in China[17] showed similar pattern of association with co-morbidities. Hypertension was the leading co-morbidity in China (39.5%), Italy (35.9%), USA (38.9%), and UK (27.8%), while in Iran diabetes was the commonest co-morbidity. Globally, diabetes was the second highest prevalent co-morbidity in 5 out of 7 countries reviewed. The presence of one or more comorbidity was associated with increased severity of the disease although not clearly associated with increased fatality rate in COVID-19 patients.[18]

Our results revealed that people having co-morbidities developed severe symptoms and had higher mortality rate compared to those with no co-morbid conditions. A total of 0.978% of HCWs with COVID-19 infection were asymptomatic. It has been reported that viral load in asymptomatic and symptomatic patient has no significant differences.[12]

In this study 77.3% of affected HCWs did not progress to severe disease that may be attributed to age; majority of HCWs were less than 50 years old. HCWs have gained sufficient knowledge to deal with viral infection at early stage. Establishment of free of

cost PCR testing with 24 hours reporting time by the Government favoured early diagnosis and treatment with a better outcome for COVID-19 HCWs and the general public.

CONCLUSION & RECOMMENDATIONS

The frontline healthcare workers are at a greater risk of COVID-19. Among healthcare workers the highest number of patients with COVID-19 were physicians. Periodic testing and booster doses of vaccine must be practiced for HCWs, especially for those who worked for the past 15 days where more than two patients with hospital associated COVID-19 infections are reported.

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ORIGINAL ARTICLE

EFFECT OF ANGER EXPRESSION ON MENTAL WELLBEING AND EMOTIONAL EXPRESSIVITY OF ORPHANS AND NON-ORPHANS

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Background: Orphan-hood is a stage that includes numerous mental and emotional problems. Lack of control on emotions puts orphans at risk of uneasiness. The aim of this study was to see the relationship between anger expressions on mental wellbeing and emotional expressions of orphans and non-orphans. **Methods:** This was a cross-sectional survey conducted from April to June 2021. Data were collected from the orphan and non-orphan adolescent students aged 13–19 years, studying in different institutes of Haripur, Pakistan. After informed consent, State Trait Anger Expression Inventory (STAXI), Short Warwick Edinburg Well-being Scale (SWEMWBS), and Emotional expressivity questionnaires were self-administered by authors and analysed on SPSS-20. **Result:** Sample size was 200 orphan and non-orphan adolescent students, with equal proportion of males and females. Anger expression was positively linked with emotional expressivity (r=0.29, p<0.001), and anger expression was negatively linked with mental wellbeing (r= -0.45, p<0.001). Orphan students scored high on anger expression (27.62±5.4) and emotional expressivity (55.28±5.79) as compare to non-orphans (23.59±5.79 and 53.31±5.99 respectively). **Conclusion:** Anger expression is positively linked with emotional expressivity and negatively linked with mental wellbeing. Girls are shyer to express their feelings compared to boys. Boys are more engaged in verbal and physical aggression than girls. Orphan boys show more aggression because they face a lot of adjustment and many other difficulties in institutions.

Keywords: Anger expression, mental wellbeing, emotional expressivity

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INTRODUCTION

An orphan is a person who does not have a living parent to look after him or her. A youngster who has lost both parents is known as an orphan.[1,2] Orphan-hood is phase exhibiting multiple psychological and emotional issues.[3] Lack of self-dedication and incapacity to take choices placed them prone to anxiety.[4] Mental health is an important aspect of overall well-being. Health is more than just the absence of disease or weakness; it is a state of total physical, mental, and social wellbeing.[5] It overall indicates the cognitive, behavioural and emotional well-being. It actually talks about how people contemplate, feel, and perform. People sometimes use the term 'mental health' to mean the absence of a mental disorder.[6] This concept has the critical effect that mental health is defined as more than the absence of mental diseases or disabilities. The term aggression refers to the range of behaviours that can cause physical and psychological harm to oneself, others, or objects in the environment.[7,8] Expressions of aggression can be made in a variety of ways, including words, mind, and body. The teenage is the most important stage of human development including the initiation of lifelong processes of physical, behavioural, cognitive and emotional growth and change. During these processes, all teenagers develop attitudes and values that guide the decisions, relationships, and understanding of specific matters. It is believed that a satisfying treatment of psychosocial well-being allows adolescents to look positively in life and make choices that do not endanger the future. Studies show that boys tend to be more physically aggressive, but verbal aggression is often referred to as relational aggression and is more common in girls.[9,10] Orphans face many psychosocial challenges, including lack of parental guidance, love for new families, care, lack of acceptance, and other risks in the immediate environment.[11] Orphans experience mental distress. Higher levels of anxiety, depression, and anger, as well as associated inactivity caused by depression; hopelessness and suicidal thoughts due to the difficulties faced after the death of the parents.

Maternal death has a poor effect at the psychosocial wellbeing of the adults even when they have crossed the 18 years threshold of orphan-hood. Yearning for moms negatively affects their coping strategies, which brings about isolation, sadness, hopelessness, loss of peace, and worry of an unsure future.[12] Psychosocial Support (PSS) is 'a continuous process that meets the physical, emotional, social, mental and spiritual needs of a child', all of which are essential for meaningful and positive human development. This includes formal and informal services dealing with their psychosocial wellbeing.

Adolescents feel very well supported by adults in their lives and religious communities. Many adolescents do not receive the social support they need from their peers and adults to effectively mitigate the

effects of stress and traumatic events in their lives.[13] As death of parent is a traumatic event that individual experiences in life and has negative effect on mental health. This study was designed to find out the relationship between anger expressions on mental wellbeing and emotional expressions of orphans and non-orphans.

METHODOLOGY

This cross-sectional study was conducted from April to June 2021 after approval from Ethics Committee of University of Haripur. After informed consent, data were collected from the participants on a specifically designed performa. Purposive sampling technique was applied. The participants comprised of orphan and non-orphan adolescent students, both male and female (n=200), aged 13–19 years. Data was collected from educational institutes of Haripur city, Pakistan. The State Trait Anger Expression Inventory (STAXI) developed by Charles D. Spielberger[14] was used to measure aggression between orphan and non-orphan adolescents. Short Warwick Edinburg Wellbeing Scale (SWEMWBS) by Rogers *et al*[15] was used to measure mental wellbeing and Emotional expressivity questionnaire[16] was used to measure emotional expressivity of orphan and non-orphans adolescents. Scales were selected on the basis of established sound reliability, STAXI (0.73–0.76), SWEMWBS (0.89) and emotional expressivity questionnaire (0.830) in relevant context.

Data were analysed using SPSS-20. Independent sample *t*-test was used to evaluate the relationship between anger expression, mental wellbeing, and emotional expressivity. Linear regression analysis was used to study the effect of anger expression on emotional expressivity and wellbeing among the students, and $p<0.05$ was considered significant.

RESULTS

Table-1 shows the psychometric characteristics of the research variables. Reliability analysis shows that the reliability coefficients of the anger expression, mental wellbeing, and emotional expression are 0.74, 0.76, and 0.86, respectively. Aggression had a significant negative correlation with psychological wellbeing (r= -0.45, $p<0.001$) and aggression had a significant positive correlation with emotional expression (r= 0.26, $p<0.001$). Mental wellbeing showed a negative correlation with emotional expression (r= -0.11, $p<0.001$).

Table-2 reveals mean, standard deviation and *t*-value for orphan and non-orphan adolescents on anger expression, mental wellbeing and emotional expressivity. Orphan students score high on anger expression (Mean=27.62, $p<0.001$) as compare to non-orphan adolescents (Mean=23.59, $p<0.001$). Non-orphans adolescents score high on mental wellbeing (Mean=26.30, $p<0.001$) as compare to orphans (Mean=22.49, $p<0.001$), and orphan adolescents significantly score high on emotional expressivity (Mean=55.28, $p<0.05$) as compare to non-orphans (Mean=53.99, $p<0.05$).

Table-3 shows mean, standard deviation and *t*-value for male and female adolescents on anger expression, mental wellbeing and emotional expressivity. Male adolescents scored significantly high on anger expression (Mean=28.10, $p<0.001$) as compare to female adolescents, (Mean=23.11, $p<0.001$). Female adolescents score high on mental wellbeing, (Mean=25.68, $p<0.001$) compare to male (Mean=23.11, $p<0.001$). Male adolescents scored high on emotional expressivity (Mean=54.66) as compare to females (Mean=53.93); but the differences were statistically non-significant ($p>0.05$).

Table-4 depicts the liner regression analysis calculated with expression of anger as predictable variable and emotional expression as outcome variable. The expression of anger was significantly positive predictor of student emotional expression (β= 0.26, $p<0.001$).The anger expression had significant negative effect on mental wellbeing (β= -0.445, $p<0.001$).

Table-1: Psychometrics properties among study variables (n=200)

Variable	n	Mean±SD	α	1	2	3
Anger Expression	200	25.6±5.94	0.74	-	-0.45*	0.29*
Mental Wellbeing	200	24.39±4.14	0.76		-	-0.11
Emotional Exp	200	54.29±5.96	0.86		-	-

*$p<0.001$

Table-2: Mean, standard deviation and *t* values for orphans and non-orphans among anger expression on mental wellbeing and emotional expressivity (n=200)

Variable	Orphans (n=100) Mean±SD	Non-orphans (n=100) Mean±SD	*t* (198)	*p*	95% CI		Cohen's d
					LL	UL	
Anger expression	27.62±5.4	23.59±5.79	5.08	0.000	2.46	5.59	0.71
Mental wellbeing	22.49±3.99	26.30±3.34	7.31	0.000	4.83	2.78	1.03
Emotional expressivity	55.28±5.79	53.31±5.99	2.36	0.019	0.32	3.61	0.33

Table-3: Mean, standard deviation and *t* value for male and female anger expression on mental wellbeing and emotional expressivity (n=200)

Variable	Males (n=100) Mean±SD	Females (n=100) Mean±SD	*t* (198)	*p*	95% CI		Cohen's d
					LL	UL	
Anger expression	28.1±5.53	23.11±5.27	6.52	0.000	3.48	6.49	0.92
Mental wellbeing	23.11±4.22	25.68±3.68	4.6	0.000	3.67	1.46	0.64
Emotional expressivity	54.66±6.31	53.93±5.59	0.86	0.388	0.93	2.39	0.12

Table-4: Liner regression analysis showing the effect of anger expression on emotional expressivity and mental wellbeing among students (n=200)

Variables		β	95% CI	
			LL	UL
Emotional Expressivity				
(constant)		47.39*	43.83	50.95
Anger Exp		0.26	0.134	0.405
R^2	0.067			
F	15.39*			
Mental Well-being				
(constant)		32.51*	30.23	34.8
Anger Exp		-0.455	-0.404	-0.23
R^2	0.203			
F	51.78*			

*$p<0.001$

DISCUSSION

In the first step reliability of the scales was ensured. The reliability conformed that the scales State Trait Anger Expression Inventory (STAXI), Short Warwick Edinburg Well-being scale (SWEMWBS) and Berkeley Expressivity Questionnaire (BEQ) had satisfactory internal consistency.

The first hypothesis 'Anger expression will negatively correlate with mental well-being of orphan male adolescents' was supported in the present study. Mental wellbeing of orphan male adolescents slightly correlates with anger expression but not very highly negatively correlate with anger expression, it may because of supportive care system for orphans in our community. The current findings are consistent with the prior research which reports that mental health and wellbeing during adolescence are strongly influenced by life experiences and relationships[17], and social support is found to be associated with mental health[18].

Second hypothesis 'Anger expression positively correlates with emotional expressivity of orphan male adolescents' was supported in our findings. Other work[19] shows that boys tend to be more physically aggressive, but verbal aggression is often referred to as relational aggression and is more common in girls. Additionally, they can struggle to find motivation for long-term goals, such as delaying immediate gratification.

Third hypothesis 'Orphan male adolescents show more anger expression than orphans female adolescents' was supported by the present study. Girls reported more emotional deprivation and abandonment than boys. Girls as children are more sensitive and fragile than boys and require more emotional care. Another common aspect of women is shame. A comparison of girls and boys showed that they did not differ in gender in terms of variable dissatisfaction, satisfaction, anxiety, fear of new situations, sleep disorders, and difficulty making friends. Nonetheless, there was a considerable difference in the magnitude of anger, desire to escape, and hope for the future.

Fourth hypothesis 'Non-orphans adolescents show less anger expression that has less effect on mental wellbeing and emotional expressivity' was also supported in the present study. Children and adolescents are taught that expressing anger or annoyance on every occasion in the home environment is not a positive behavior.[20] Orphanage children had more mental health problems than foster children. Most studies have shown that orphans are more likely to experience psychosocial problems than non-orphan peers.[11,12,20] Gender and age were not significantly associated with mental wellbeing. Parental involvement is primarily related to the psychological adaptation of the child.[21]

CONCLUSION

Anger expression is positively linked with emotional expressivity and negatively linked with mental well-being. Girls are shyer to express their feelings as compared to boys. Boys are more engaged in verbal and physical aggression than girls. Orphan boys show more aggression because they face a lot of adjustment and many other difficulties in institutions. Non-orphan adolescents show less aggression; it may be because they are taught under the supervision of both parents not to express their anger.

IMPLICATIONS OF THE STUDY

The current study findings highlighted the need of developed supportive care system in our society. Mental well-being of orphan adolescents should be improved by providing proper support and care systems.

LIMITATION AND SUGGESTIONS

This was a single centre study. Similar work should also be done in other areas of the country, and in other social settings too.

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