

ORIGINAL RESEARCH

Development of omega-3 rich eggs through dietary flaxseed and bio-evaluation in metabolic syndrome

Hira Shakoor¹ | Muhammad Issa Khan¹  | Amna Sahar¹  |
Muhammad Kashif Iqbal Khan²  | Furukh Faiz³ | Hafiz Basheer Ahmad⁴

¹National Institute of Food Science and Technology, University of Agriculture Faisalabad, Pakistan, Faisalabad

²Department of Food Engineering, University of Agriculture Faisalabad, Faisalabad, Pakistan

³Department of Agriculture and Food Technology, Karakoram International University, Gilgit, Pakistan

⁴Ayub Agriculture Research Institute, Faisalabad, Pakistan

Correspondence

Muhammad Issa Khan, National Institute of Food Science and Technology, University of Agriculture Faisalabad, Pakistan-38040.
Email: drkhan@uaf.edu.pk

Funding information

University of Agriculture

Abstract

An egg is a nutrient-dense food that contains protein, fats, vitamins, and minerals. It is proven that the consumption of eggs influences serum lipid concentration. Therefore, a study was conducted to investigate the effect of normal and omega-3 eggs on serum lipids profiles. Lipids were extracted from egg yolks and analyzed for fatty acids content. The present research is a crossover study design in which 20 participants were recruited randomly, and all subjects received three treatments: no eggs, omega-3 eggs, and normal eggs. However, fasting blood was drawn at baseline and the end of each diet period and analyzed for serum lipids, blood glucose, and insulin level. Omega-3 egg treatment showed reduction in the serum total cholesterol by 16.57 mg/dl ($p < .001$), triglyceride by 17.48 mg/dl, and increase in HDL cholesterol concentration by 0.48 mg/dl ($p < .001$) as compared to no-egg. A significant ($p < .05$) reduction in blood pressure by 8.34/8.67 mm/Hg and insulin level was observed due to omega-3 egg consumption which indicates that omega-3 fatty acids improve insulin sensitivity. On the other hand, regular egg intake elevates serum total cholesterol and triglycerides concentration but decreases blood pressure. It was concluded that omega-3-enriched egg consumption had a positive effect on the serum lipid profile and blood pressure of patients with metabolic syndrome as compared to normal eggs.

KEYWORDS

high-density lipoprotein, low-density lipoprotein, metabolic syndrome, omega-3 fatty acid, very low-density lipoprotein

1 | INTRODUCTION

A metabolic syndrome is a group of metabolic risk factors such as obesity, glucose intolerance, dyslipidemia, and insulin intolerance, that affects approximately 20%–25% of the adult population in the world (Bhowmik et al., 2015). Diet plays a vital role in protecting the body from these metabolic anomalies, while functional food provides additional health benefits besides basic nutrients (Khan, Khan, et al.,

2017). Different bioactive components of the diet are considered to regulate metabolic syndrome, including phospholipids, omega-3 fatty acids, arginine, folate, some minerals, and so forth (Fuller, Sainsbury, Caterson, & Markovic, 2015). Among these healthy diet components, omega-3 fatty acids, such as alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), are proven to have a potential mechanistic approach against multiple risk factors of metabolic syndrome including hyperlipidemia, hypertension, high blood

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glucose, hepatic steatosis, and endothelial dysfunction (Dasarathy et al., 2015). An egg can contribute 72 kcal and has some healthy elements, including protein, bioactive lipids (phospholipids), some fat-soluble vitamins (A, D, E, and K), vitamin B complex, and minerals (Andersen, 2015; Larsson, Akesson, & Wolk, 2015). Enrichment of eggs with omega-3 contents is inexpensive way to make it readily available for everyone. Addition of flaxseed in hen's diet increases the amount of omega-3 fatty acids, *that is*, docosahexaenoic acid (DHA) and alpha-linolenic acid (ALA). These omega-3-enriched eggs contain up to five times more omega-3 fatty acid constituents compared to normal eggs (Egg Nutrition Council, 2014). In order to reduce the cardiometabolic risk, it is essential to have a diet that is rich in omega-3 fatty acids (Yashodhara et al., 2009). Evidence showed that omega-3 fatty acids are involved in reverse cholesterol transport, improve vascular compliance, and decrease arterial stiffness. Hence, it reduces the risk of atherosclerosis and insulin resistance (Blesso, Andersen, Barona, Volek, & Fernandez, 2013; Papanikolaou, Brooks, Reider, & Fulgoni, 2014; Tousoulis et al., 2014). The omega-3 fatty acids are present in eggs which help in reducing the level of cholesterol, triglycerides, and the particle size of low-density lipoprotein (LDL) by converting them to the less atherogenic elements (Lewis, Schalch, Scheideler, 2000; Lewis, Seburg, & Flanagan, 2000). A 13% triacylglycerol reduction was found in a group that consumed omega-3 eggs, while 18% triacylglycerol reduction was observed in group consuming walnuts (Burns-Whitmore, Haddad, Sabate, & Rajaram, 2014). Moreover, egg phospholipids help in the reduction of lipid absorption, C-reactive protein (CRP), and the alpha factor of tumor necrosis in plasma (Blesso, 2015; Compos et al., 2016).

Unhealthy dietary habits and sedentary lifestyle increase the risk of metabolic syndrome, diabetes, hypertension, and cardiovascular disease (Fuller et al., 2015). To deal with the increasing risk of diseases, the present study was conducted to evaluate the effect of omega-3 eggs in patients with metabolic syndrome.

2 | MATERIALS AND METHODS

2.1 | Production of omega-3-enriched eggs

The study was undertaken at National Institute of Food Science and Technology, University of Agriculture Faisalabad. The omega-3-enriched eggs were produced under the project of "Development of conditioned (omega-3-rich) meat and eggs through modification in feed ingredients." Omega-3-enriched eggs were produced by adding 20% flaxseed to the hens' diet. Omega-3 in the hen diet boost omega-3 in egg yolk up to 220 mg which is equal to 100 g lean fish (Hargis & Van Elswyk, 1993).

2.2 | Fatty acids profile of eggs

Lipids from egg yolks were extracted by following the method described by Folch, Lees, and Sloane-Stanley (1957). Esters of fatty

acids were prepared and analyzed using gas chromatography according to the (Ce if-96) method given in AOCS (American Oil & Chemist Society, 1998).

2.3 | Subjects

This study was a randomized crossover (3×3 Latin square), free-living intervention experiment in which subjects were blinded to egg treatments. Twenty-one subjects aged 30–40 years old, diagnosed with metabolic syndrome, were included in this study. The selection criteria for patients were according to the International Diabetes Federation (IDF) consensus which states obesity: waist circumference 94 cm or more for men and 80 cm or more for women, waist-to-hip ratio (WHR) 0.90 cm or above for males and 0.85 or above for females, and body mass index (BMI) 30 or above. Hypertension: systolic BP measurement of 130 mmHg or more and diastolic BP measurement of 85 mmHg or above. High blood glucose level: fasting blood glucose of 100 mg/dl (5.6 mmol/L) or more, but the patient is not suffering from type 2 diabetes mellitus. Dyslipidemia: blood triglycerides level of 150 mg/dl (1.7 mmol/L) or more, or may use cholesterol-lowering medicine such as statins. Dyslipidemia: low level of high-density lipoprotein (HDL) cholesterol which is less than 40 mg/dl (1.04 mmol/L) for men and less than 50 mg/dl (1.3 mmol/L) for women, or the use of cholesterol-lowering medicine like statins (Bhowmik et al., 2015). Patients with egg allergy, insulin-dependent diabetes mellitus, heart diseases, stroke, acute or chronic insufficiency, cancer, liver failure, and endocrine disorders were excluded from the study.

2.4 | Treatment plan

Three treatments were used in this study: no eggs, two boiled omega-3 eggs per day, and two boiled normal eggs per day at breakfast. Subjects were blinded to the egg treatment and randomly assigned to the treatment in a manner consistent with the Latin square protocol. The study design was a crossover (3×3 Latin square) such that each subject received each of the three treatments. Before the study, the baseline value of serum lipid profile, blood pressure, glucose, and insulin level was recorded, and every individual served as his or her own control. Each treatment was conducted for 5 weeks, with a five-week washout period between treatments, in order to minimize the possible carryover effects. Exercise and medication were maintained during the study period.

2.5 | Anthropometrics and blood pressure assessment

Subjects' weight (kg) and height (m) were measured to calculate their body mass index (BMI) (kg/m^2), as described by Keys, Fidanza,

Karvonen, Kimura, and Taylor (1972). Waist and hip circumference of the subjects was estimated according to the criteria described by Skorkowska-Telichowska (2016). Blood pressure was monitored after 15 min of rest by a standardizing mercury manometer placed on the midupper arm of the subject following the method of Oh, Ryue, Hsieh, and Bell (1991).

2.6 | Plasma lipids profile

Fasting blood samples of the subjects, collected by syringe at the beginning of the experiment as a baseline value and at the end of each treatment period, were analyzed for serum lipids. Serum lipid profile, including serum total cholesterol and triglycerides, were analyzed by the enzymatic method of Allian, Poon, Chan, Richmond, and Fu (1974) and Nagele et al. (1984). High-density lipoprotein (HDL) cholesterol was measured directly after the precipitation of other lipoproteins by dextran sulfate (Warnick, Nguyen, & Albers, 1985). Low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) were calculated. Plasma LDL and VLDL cholesterol concentrations were measured using the Friedewald, Levy, and Fredrickson (1972) equation as follows:

VLDL = TRIG/5 and LDL = TC - (VLDL + HDL) as mentioned in Nijke, Faridi, Dutta, Gonzalez-Simon, and Katz (2010).

The normal range of total cholesterol is <200 mg/dl, triglyceride is 50–150 mg/dl, HDL is 35–60 mg/dl, and LDL is <150 mg/dl.

2.7 | Glucose and insulin level

The blood sugar level of the subjects was determined using ACCU-CHEK (Dacus, Schulz, Averill, & Sibai, 1989), whereas the insulin level was measured following the method of Ballesteros et al. (2015).

2.8 | Statistical analysis

The data were subjected to statistical analysis using SPSS 23 and Minitab 17. The crossover (3 × 3 Latin square) design was used to determine the significance level. When the trend was significant, the difference among treatments was analyzed by the Tukey HSD test and $p < .05$, which was adjusted for multiple comparisons.

3 | RESULTS

Among the 21 eligible participants at start of the study, one patient was dropped out due to time constraints and lack of motivation to continue the study. About 20 individuals including 11 men and 9 women completed all three treatments. Clinical trials were conducted on patients with metabolic syndrome to determine the impact of eggs on the serum lipid profile. However, the results regarding each parameter are summarized as follows.

TABLE 1 PUFA composition (%) of normal and omega-3-rich egg yolks

Fatty acid	T1 (%)	T2 (%)	p value
C18:3 (n = 3)	1.16 ± 0.04 ^b	7.39 ± 0.08 ^a	.000**
C18:2 - Cis (n = 6)	7.71 ± 0.19 ^b	9.61 ± 0.07 ^a	.004*
C20:5 (n = 3)	0.76 ± 0.07 ^b	1.53 ± 0.03 ^a	.003*
C22:6 (n = 3)	0.33 ± 0.03 ^b	6.20 ± 0.03 ^a	.000**
C22:5 (n = 3)	0.06 ± 0.002 ^b	0.33 ± 0.006 ^a	.000**

Note: All the values are mean ± SE; n = 20, means within the same row having different superscripts are significantly different ($p < .05$).

T1, normal eggs, T2, omega-3 fatty acid.

3.1 | Fatty acids composition of eggs

Gas chromatography was used to analyze the FAME of eggs and identify different types of fatty acids including C18:3, C18:2, C20:5, C22:6, and C22:5. The 20% flaxseed in the hens' diet showed an increase in the level of polyunsaturated omega-3 fatty acids C18:3 by 7.39%, C20:5 by 1.53%, C22:6 by 6.20%, C22:5 by 0.33%, and a slight increase in omega-6 fatty acid by 9.61% compared to the control eggs. As shown in Table 1, in modified eggs (omega-3 egg), the percentage of omega-3 and omega-6 fatty acid was highly significant as compared to normal eggs.

3.2 | Anthropometric measurements

Participants included in the study were overweight because their BMI and WHR were higher than normal ranging from 28 to 30 and 0.92 to 0.95, respectively. Consumption of normal and omega-3 eggs does not have significant effect on BMI and WHR of patients with metabolic syndrome (Table 2.)

3.3 | Serum lipid profile

The comparison of normal egg and no-egg treatments with omega-3 egg treatment showed a significant decrease in total cholesterol from 46.14 to 16.57 mg/dl, respectively ($p < .001$). Taking two omega-3 eggs in breakfast reduce total cholesterol and triglycerides by 12.5% and 10.5%, respectively as shown in Table 3, whereas normal egg consumption increases total cholesterol to 227.523 mg/dl. Moreover, omega-3 eggs resulted in increase of HDL level by 9.3% compared to normal eggs (Mutungi et al., 2008). On the other hand, no change was seen in treatment of normal eggs. A similar trend was observed in serum LDL and VLDL cholesterol concentration, in which a slight reduction was observed due to omega-3-enriched eggs compared to no-egg and normal egg consumption. However, the result is statistically insignificant.

	No-egg	Omega-3 egg	Normal egg	p value
WHR	0.920 ± 0.016	0.938 ± 0.016	0.920 ± 0.016	.161
BMI	30.210 ± 1.044	29.933 ± 1.044	28.904 ± 1.044	.274

Note: All the values are mean ± SE; *n* = 20, means within the same row having different superscripts are significantly different by Tukey's HSD (*p* < .05).

Abbreviations: BMI, body mass index; WHR, Waist-to-hip ratio.

3.4 | Blood pressure, glucose, and insulin level

The lowest value of blood pressure (82/122 mm/Hg) was observed in omega-3 egg treatment, and the highest value of (90/130) mm/Hg was seen in the no-egg treatment. A significant (*p* < .001) reduction of blood pressure by 8.34/8.67 mm/Hg due to omega-3 egg consumption. Interestingly, improvement in blood pressure was also seen in normal egg treatment (128/85 mm/Hg) compared to the no-egg, as shown in Table 4.

Omega-3 eggs improve glucose and insulin levels by 4% and 14%, respectively, indicating improvement in insulin sensitivity. The insulin level of patients with metabolic syndrome exhibited a significant (*p* < .05) difference among all three groups.

4 | DISCUSSION

The nutraceutical value and health benefits of eggs can be enhanced by adapting appropriate feeding strategies in poultry as well as by

developing designer eggs like omega-3-enriched eggs (Laudadio et al., 2015). Feed modification of the hens elevated the omega-3 contents in the egg yolk. However, a previous study investigated that feed modification of hen with flaxseed increases ALA to 200 mg per egg and raises the amount of DHA content up to 90 mg per egg (Lemahieu et al., 2015). Our findings showed that the addition of 20% flaxseed in hens' diet increases linolenic acid, EPA, and DHA in egg yolks significantly as compared to normal eggs, which is similar to the findings of Al-Nasser et al. (2011).

Anthropometric measurements are an essential parameter for the identification of individuals with metabolic syndrome. Addition of two boiled eggs (omega-3 and normal) in diet did not significantly affect BMI and WHR but previous studies showed a change in anthropometric measurement due to egg consumption (Blesso, Andersen, Barona, Volek, et al., 2013; Skorkowska-Telichowska et al., 2016). Nevertheless, polyunsaturated fatty acid (PUFA) serves as good fats for human health; therefore, increasing PUFA contents in the egg yolk helps to decrease the bad cholesterol (Fraeye et al., 2012). Our finding reported a reduction in serum cholesterol and

TABLE 2 Anthropometric measurements at end of each treatment

TABLE 3 Serum lipid level of individuals at end of each treatment

	No-egg	Omega-3 egg	Normal egg	p value
Cholesterol (mg/dl)	197.952 ± 8.527 ^b	181.381 ± 8.527 ^a	227.523 ± 8.527 ^c	.001**
Triglycerides (mg/dl)	169.952 ± 7.489	152.476 ± 7.489	187.333 ± 7.489	.082
HDL (mg/dl)	46.619 ± 2.790 ^a	47.095 ± 2.790 ^a	42.904 ± 2.790 ^b	.010*
LDL (mg/dl)	139.333 ± 16.500	98 ± 16.500	124.366 ± 16.500	.072
VLDL (mg/dl)	35.900 ± 5.242	31.166 ± 5.242	41.600 ± 5.242	.180

Note: All the values are mean ± SE; *n* = 20, means within the same row having different superscripts are significantly different by Tukey's HSD (*p* < .05).

	No-egg	Omega-3 egg	Normal egg	p value
Diastolic (mm/Hg)	90 ± 1.708 ^c	81.667 ± 1.708 ^a	85 ± 1.708 ^b	.001**
Systolic (mm/Hg)	130 ± 1.849 ^b	121.667 ± 1.849 ^a	128.333 ± 1.849 ^b	.001**
Glucose (mg/dl)	117.833 ± 3.237	111.833 ± 3.237	114 ± 3.237	.845
Insulin level (u IU/ml)	20.083 ± 2.311 ^b	18.700 ± 2.311 ^a	24.667 ± 2.311 ^c	.058*

Note: All the values are mean ± SE; *n* = 20, means within the same row having different superscripts are significantly different by Tukey's HSD (*p* < .05).

TABLE 4 Blood pressure, glucose and insulin level at the end of each treatment

triglyceride concentration by 12.5% and 10.5%, respectively, due to omega-3 egg consumption. Omega-3 fatty acids and phospholipids present in omega-3 egg yolk help to alter the mobilization and metabolism of cholesterol and hence decrease cholesterol level (Jiang, Noh, & Koo, 2001; Khan, Hegde, Wagh, Khan, & Jamadar, 2017; Noh & Koo, 2003). However, normal eggs are rich in cholesterol and can contribute 32% of total dietary cholesterol (Hu et al., 1999). That is why consumption of normal eggs can increase total serum cholesterol as compared to no-egg (Chakrabarty et al., 2002; Rouhani, Rashidi-Pourfard, Salehi-Abargouei, Karimi, & Haghghatdoost, 2017; Severins, Mensink, & Plat, 2015). Weggeman et al. meta-analysis of clinical trials observed that 100 mg dietary cholesterol from eggs increases plasma total cholesterol by 0.057 mmol/L (Weggemans, Zock, & Katan, 2001).

National Lipid Association (NLA), American College of Cardiology/American Heart Association (ACC/AHA) recommends omega-3 fatty acid as the first-line option for patients with hypertriglyceridemia to decrease triglyceride level (Jacobson et al., 2014; Stone et al., 2014). Omega-3 egg treatment reduces triglyceride level by 17 mg/dl in patients with metabolic syndrome compared to the no-egg. However, normal eggs enhanced serum triglyceride level. Likewise, evidence proved that linolenic acid-enriched eggs decreased triglycerides concentration, thereby reducing the risk of cardiovascular diseases (Ferrier et al., 1992; Lewis, Schalch, Scheideler, 2000; Lewis, Seburg, et al., 2000), and these findings are in line with our results. Further, omega-3 egg consumption in hypertriglyceridemic participants for 6 weeks lowers triglycerides and increases HDL cholesterol as compared to regular eggs (Maki et al., 2003). Evidence showed that omega-3-enriched eggs enhanced HDL cholesterol concentration (Andersen et al., 2013; Farrell, 1998; Lee, Lewis, Scheideler, & Carr, 2003; Techakriengkrai, Klangjareonchai, Pakpeankitwattana, Sritara, & Roongpisuthipong, 2012). Undoubtedly, omega-3 PUFA increases systemic lipoprotein lipase activity that has hypotriacylglycerolaemic effect. For instance, omega-3 fatty acids decrease the availability of circulating triglycerides that eventually reduce the hepatic synthesis of LDL and VLDL cholesterol (Buckley, Shewring, Turner, Yaqoob, & Minihaue, 2004). In the present study, normal egg consumption slightly decreases LDL cholesterol as compared to no-egg, but the notable reduction was observed with omega-3 egg consumption. VLDL did not show a significant trend with egg consumption, and its value was highest among normal egg treatments. The results of our studies are similar to the finding of Techakriengkrai et al. (2012). Similarly, it demonstrated that daily egg consumption showed no significant change in LDL cholesterol of the participants.

In the present study, consumption of two omega-3 eggs reduced diastolic and systolic by 13.6% and 6%, respectively. It is proven that omega-3 fatty acids help to improve vascular health and arterial stiffness which are associated with metabolic syndrome (Chong, Lockyer, Saunders, & Lovegrove, 2010). Previous studies found that omega-3 eggs decrease blood pressure (Djousse, Gaziano, Buring, & Lee, 2009; Oh et al., 1991; Stupin et al., 2018). Another condition of metabolic syndrome is high blood glucose level. The present study reported that omega-3 eggs and normal eggs do not significantly affect the

glucose level. Similar results were found in the previous clinical trials that showed no improvement in blood glucose level with daily egg consumption in patients with type 2 diabetes (Djousse et al., 2009; Njike et al., 2010). Contrarily, Pourafshar et al. findings showed a reduction in glucose level (Pourafshar et al., 2016). However, in our study, patients with metabolic syndrome who consumed omega-3 eggs had improved insulin levels by 14%, and this is statistically significant compared to other treatments. In another research, Samimi, Jamilian, Asemi, and Esmailzadeh (1988) examined the effect of omega-3 egg consumption on blood glucose and insulin level in a female with gestational diabetes over a 6-week period which proved the positive effects of omega-3 on insulin resistance but had no effect on glucose level which is in line with our findings. Furthermore, evidence showed increase in insulin sensitivity by consumption of an egg-based breakfast, which proved the beneficial effect of eggs (Clayton et al., 2015).

The present study is a free-living intervention study in which subjects were not forced to control their diet. However, the previous studies were controlled feeding trial, which mainly follow the dietary guidelines of National Cholesterol Education Program (NCEP) diet (that suggests 30% of total calories come from fat, 10% from saturated, and less than 300 mg/day of dietary cholesterol) and carbohydrate-restricted diet (Blesso, Andersen, Barona, Volk, et al., 2013; Lewis, Schalch, Scheideler, 2000; Lewis, Seburg, et al., 2000). Further, the effect of omega-3 eggs along with normal eggs on all parameters of the metabolic syndrome was observed and a positive association was identified between egg consumption and human health. In contrast to prior studies that have focused on the lipid parameters, we also measured the impact of egg treatments on blood pressure, glucose level, and insulin level and found that omega-3 eggs were good for hypertensive and prediabetic patients. Although the limitation of this study was that a small number of patients were recruited, which may be insufficient to evaluate the change in lipid profile, blood pressure, and glucose level, hence, further studies are needed to confirm these findings using a larger sample size in a real-life setting.

5 | CONCLUSIONS

In light of sustained uncertainties and absence of observational evidence about the influence of omega-3 egg consumption on serum lipid profile, the present study was conducted to identify the potential benefits of eggs. Omega-3 eggs are nutritious food which occupies an essential place in the food chain, and it also acts as a functional food. Advancements in technology and modern living style increase the consumption of saturated fats day by day and sedentary lifestyle. Replacement of saturated fats with healthy monounsaturated and polyunsaturated fatty acids can help to reduce the risk of diseases. Production of omega-3-enriched eggs is a good strategy to include omega-3 fatty acids in the diet at low cost. Consumption of omega-3-enriched eggs has a positive potential toward the reduction of cholesterol, triglycerides, LDL cholesterol, blood pressure, and increase HDL cholesterol concentration. On the other hand, normal eggs elevate serum cholesterol, triglyceride, and

VLDL level due to high content of cholesterol in the yolk. Therefore, omega-3-enriched eggs are proved to be beneficial for heart health and for the reduction of many life-threatening diseases.

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CONFLICT OF INTEREST

The authors declare that they do not have any conflict of interest.

AUTHORS' CONTRIBUTIONS

HS, MIK, and AS conceived and designed the study. HS conducted the human study, analyzed the fatty acid contents in eggs and biochemical test of patients, and prepared the results and figures. MIK and AS supervised the practical work. HS wrote and finalized the manuscript. AS reviewed the manuscript for possible improvement. MKIK and HBA checked English grammar, statistical analysis, text, organization, and typesetting of the paper. All authors read and approved the final manuscript.

ETHICAL APPROVAL

Experiments were conducted in accordance with the declaration of Helsinki principles for human subjects. The protocol was approved by the Institutional Bioethical Committee, National Institute of Food Science and Technology, and University of Agriculture Faisalabad, Pakistan. All subjects were briefed about study, and written consent was obtained from all study participants.

ORCID

Muhammad Issa Khan  <https://orcid.org/0000-0002-3241-1248>

Amna Sahar  <https://orcid.org/0000-0002-9299-4000>

Muhammad Kashif Iqbal Khan  <https://orcid.org/0000-0003-0090-3608>

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