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Qualitative estimation and quantitative confirmation of micro elements in bread wheat genotypes by multivariate study

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Micronutrient scarcity is a universal challenging apprehension and roughly half of the global population has micro elements deficiency primarily iron and zinc. Bio-fortified wheat being natural rich source of minerals will be beneficial for diminishing prevalence of iron and zinc malnutrition among masses. A panel of fifty bread wheat genotypes from national wheat breeding program were qualitatively tested and quantitatively verified for different micro elements via cheaper qualitative and standard techniques. Results showed that a simple association uncovered highly affirmative significant correlation of zinc with all micro elements. Magnesium linked positively highly significant with calcium whilst calcium positively significantly allied with iron. In addition, the remaining micro nutrients depicted positive association with one another. PC1 have eigen value >1 while PC2 and PC3 were at par to 1 which added 69.9% of total disparity. The deliberated traits disjointed into 5 clusters which hold 8, 14, 12, 13 and 3 genotypes. On biplots, the genotypes those contiguous jointly were perceptible as being similar when appraised on five elements while elements which were more distant were more diverse from other genotypes. As regards quantitative confirmation, all 50 genotypes dropped in diversified ranges for iron and zinc content. Dithizone and Prussian blue procedures were found to be effective, novel and cheaper qualitative techniques for zinc and iron assessment in massive wheat genotypes. The genetic stock examined can be used as a valuable yardstick for breeding strategies (bio-fortified wheat) and wheat processing industry for enterprising growing malnutrition and magnification encounters of food safety.

Keywords: Quantitative, qualitative, corroboration, minerals, multivariate, bread wheat

INTRODUCTION

Micronutrient deficiencies severely affect more than two billion people globally which ultimately lead to death if left unattended (Hannah and Max, 2019). Worldwide most frequent micronutrient deficiencies are folic acid, vitamin A, iodine, iron and zinc (Bailey et al., 2015). Many physiological and developmental abnormalities in human body are associated with zinc deficiency (Osredkar, 2011) which is more prevalent in developing countries (Wessells and Brown, 2012). Severe deficiency of iron and zinc, individually and in combined form, cost deaths in large number especially in children, as children and pregnant women are more prone to nutritional deficiencies due to metabolic imbalances and weaker immune system (Black et al., 2013; Gakidou et al., 2017). Wheat is staple food in the temperate climate and is abundantly consumed in developing countries. It contains low concentration of bio-available iron and zinc and is therefore contributes to iron and zinc deficiency where it is being used as staple crop. There are two reasons that cause low iron and zinc concentration in wheat; one is genetically less presence of these minerals and second one is availability of high phytate content in grain bran portion which binds the minerals and makes them unavailable. However, deficiency of these minerals in wheat grain can be overcome sustainably through application of bio-fortification and transgenic technique by converting the endospermic portion into the minerals sink (Balk et al., 2019). Moreover, minerals bioavailability may also be significantly enhanced by fermentation and micro grinding processes (Aslam et al., 2018).

Recommended Dietary Allowance (RDA) for iron and zinc in adults is 8-11 mg per day (Institute of Medicine, 2001) while 100 gram of bread wheat has 3.19 mg iron and 2.65 mg zinc.

Soil application of zinc at a dose of 50 kilogram per hectare effectively increased kernel zinc content only in the zinc deficient soils. Foliar zinc application noticeably enhanced zinc concentration (11-22 ppm) of both whole kernel and its various components particularly under high nitrogen soil application while combination of foliar and soil zinc appliance increased zinc content up to 27 ppm but use of foliar zinc spray twice (booting and milky phase) along with high soil nitrogen fertilization, significantly enhanced grain zinc content from 28-58 ppm (Cakmak et al., 2010).

Determination of minerals by Atomic absorption spectrophotometer technique (which is not available with most of labs working on wheat quality) is costly, laborious and time consuming. However, staining techniques based on qualitative grounds are cost effective, cheaper and more reliable approaches for iron and zinc content estimation. These qualitative staining techniques have been found effective for the rapid screening of large number of pearl millet genotypes (Velu et al., 2008). The core objective of the present study was to find out the quick and economical technique for determination of minerals, particularly iron and zinc, in huge wheat genetic stock to identify superior genotypes for stated minerals which will be suggested as potential material to be included in breeding program for nutrition enhancement.

Moreover, fortified wheat products also being costly are not in easy access to a large segment of poor human population and their effectiveness is also a question mark (Lalani et al., 2019). Therefore, bio-fortified wheat being natural rich source of minerals will be beneficial for diminishing prevalence of iron and zinc malnutrition among masses through the production and consumption of various bio-fortified wheat products such as flat bread (chapati), fermented bread and other bakery items to overcome malnutrition challenges and strengthened national food security.

MATERIALS AND METHODS

Genotypes (n=50) from national wheat breeding program were collected (Table 1) and analyzed for five micronutrients (iron, zinc, calcium, magnesium and copper). Among these minerals, iron and zinc were qualitatively tested by the cheapest and quick staining methods of Prussian blue technique and Dithizone staining, respectively (Ozturka et al., 2006) and further quantitatively confirmed by Atomic Absorption Spectrophotometer technique using AOAC Method No. 985.35 (Model: 969, Unicam Limited, United Kingdom); Furnace with temperature range 250-600±10ºC and Furnace Auto-Sampler (Horwitz & Latimer, 2006).

Prussian blue technique for the qualitative assessment of iron in Wheat

Ten gram (10g) Potassium ferrocyanide (2%) was taken in a flask and dissolved in distilled water using vortex mixer to get uniform mixture. Then volume of the flask was made up to 500 ml with distilled water and the resultant material was shifted into brown glass bottle for storage. Similarly, 10 ml concentrated hydrochloric acid (2%) was taken in a volumetric flask and mixed with distilled water up to 500 ml volume and the solution was transferred into a brown glass bottle for storage. For each sample, fresh Prussian blue solution was prepared by taking 5 ml each from both solutions. Now 0.5 g wheat flour was taken in the petri dish and 10 ml fresh Prussian blue solution was added in it and color of the samples was recorded after 10 minutes. Color strength was noted by visual observation on a scale of 1-3 indicating 1 as low blue color, 2 as medium blue and 3 as high/strong blue.

Dithizone staining technique for the qualitative assessment of zinc in Wheat

Five hundred mg (500mg) Dithizone (1, 5-diphenyl thiocarbazone) of 99 % purity was taken in a beaker and dissolved in one liter methyl alcohol with the help of mixer until uniform mixing. Then I g whole wheat flour was taken in borosilicate glass tube and 5 ml freshly prepared Dithizone solution was added in the tube. The samples were uniformly mixed for two minutes through vortex mixer for staining purpose. These samples were then kept in incubator at room temperature for 15 minutes so that maximum color intensity was obtained. Color development was visually recorded on a scale of 1-3 in which 1 showed less red color, 2 as medium red color and 3 as high/ strong red color.

Table 1: Genotypes studied and their parentages

Genotypes No.	Parentages	Genotypes No.	Parentages
1	ZINCOL-16 (LOCAL CHECK)	**26**	HOLO/BORL14//VALI
2	BAJ #1	**27**	REH/HARE//2*BCN/3/CROC_ 1/AE.SQ (213)//PGO/4/..
3	KACHU #1	**28**	FRET2/TUKURU//FRET2*2/3 /T.SPELTA PI348530/4/..
4	ZINCSHAKTHI	**29**	KATERE/3/QUAIU #1 /SOLALA//QUAIU #2/4/..
5	MAYIL	**30**	KATERE/2*BORL14
6	DANPHE #1*2/3/T.DICOCCON\ PI94625/AE.SQ (372)//..	**31**	KATERE/BORL14/3/ WBLL1*2/KURUKU//..
7	QUAIU #1/3/T.DICOCCON PI94625/AE.SQ (372)//..	**32**	KATERE/BORL14/3/W BLL1*2/KURUKU//..
8	QUAIU #1/3/T.DICOCCON P I94625/AE.SQ (372)//..	**33**	CROC_1/AE.SQUARROSA (210)//PBW343*2/..
9	WHEAR/KUKUNA/3/C80.1/3* BATAVIA//2*WBLL1/..	**34**	SHAKTI/2*BORL14
10	MANKU/ZINCOL	**35**	VALI/MAYIL
11	MANKU/ZINCOL	**36**	WHEAR/KUKUNA/3 /C80.1/3*BATAVIA//..
12	KOKILA/BOKOTA	**37**	WHEAR/KUKUNA/3 /C80.1/3*BATAVIA//..
13	ZINCOL/VALI	**38**	MAYIL/ZINCOL
14	ZINCOL/VALI	**39**	HOLO/VALI
15	PAURAQ//RL6043/4*NAC/3 /QUAIU #1/SOLALA//..	**40**	VILLA JUAREZ F2009/3/T .DICOCCON PI94625/..
16	DANPHE #1*2/3/T.DICOCCON PI94625/AE.SQ (372)//..	**41**	VILLA JUAREZ F2009/3/T .DICOCCON PI94625/..
17	VALI*2/6/WHEAR/KUKUNA /3/C80.1/3*BATAVIA//..	**42**	ZINCOL/3/QUAIU #1/ SOLALA//QUAIU #2
18	VALI/6/2*WHEAR/KUKUNA /3/C80.1/3*BATAVIA//..	**43**	REH/HARE//2*BCN/3/ CROC_1/AE.SQ (213)//..
19	VALI/MAYIL/6/WHEAR/KUKUN A/3/C80.1/3*BATAVIA//..	**44**	CROC_1/AE.SQUARROSA (210)//PBW343*2/..
20	VALI/MAYIL/6/WHEAR/KUKUN A/3/C80.1/3*BATAVIA//..	**45**	QUAIU #1/3/T.DICOCCON PI94625/AE.SQ (372)//..
21	WHEAR/KUKUNA/3/C80.1/3 *BATAVIA//2*WBLL1/4/..	**46**	QUAIU #1/3/T.DICOCCON PI94625/AE.SQ (372)//..
22	COAH90.26.31//KIRITATI/W BLL1/3/KIRITATI/2*WBLL1/..	**47**	QUAIU #1/3/T.DICOCCON PI94625/AE.SQ (372)//..
23	QUAIU #1/3/T.DICOCCON PI9 4625/AE.SQ (372)//3*PASTOR/..	**48**	HGO94.7.1.12/2*QUAIU#1/6 /CHIH95.2.6/4/..
24	QUAIU #1/3/T.DICOCCON PI9 4625/AE.SQ (372)//3*PASTOR..	**49**	VILLA JUAREZ F2009/3/T .DICOCCON PI94625/..
25	MAYIL/ZINCOL//ITP40/AKURI	**50**	68.111/RGB-U//WARD/3 /AE.SQ (321)/4/..

The data in two replications was subjected to basic statistics, correlation analysis, cluster analysis and principal component analysis using software package of SPSS version 12 and STATISTICA version 5.0 (Sneath and Sokal, 2014).

The cluster analysis was performed using K-means clustering while dendrogram based on elucidation distances was developed by Ward's method.

RESULTSAND DISCSSION

The Analysis of variance (ANOVA) for all dignified traits was carried out (Table 2). The means analysis of variance revealed highly significant results ($P \leq 0.01$) which imply the manifestation of adequate inconsistency among genotypes for the traits measured. These fallouts were confirmatory with the verdicts of Kondou et al., (2016) who also found highly significant differences for four (04) mineral elements studied. Zinc presented a highly positive significant correlation with all elements except Cu. Furthermore, it was found to be positively significant only which advocated that this element is vital and can be used as an effective benchmark for screening genotypes for breeding program.

The principal component analysis imitates the status of the biggest donor to the entire deviation at each axis of discrepancy (Sharma, 1998). Among 50 genotypes, the minimum (70.5ppm) and maximum (948.5ppm) value of Fe was recorded for genotype 11 and 48 with average value of 306.43ppm. The genotypes 3 and 43 exhibited minimum (35.74ppm) and maximum (65.56ppm) value for Zn contents in an average of 43.18ppm. The Ca contents were found to be in the range of 0.074 (genotype 5) to 0.116% (genotype 27) with an average of 0.0908%.

Likewise, the range observed for Mg and Cu was 0.136 to 0.171% and 8.86 to 29.15ppm in genotypes 2 and 15 with an average of 0.153% and 13.46ppm, respectively. Choi et al., (2013) also found a wide range of these inorganic elements in Korean wheat. A simple correlation "all against all" exposed significant correlation amongst 5 elements studied in 50 genotypes (Table 3).

The studied traits fragmented into 5 clusters. The cluster 1, 2, 3, 4 and 5 contained 8, 14, 12, 13 and 3 genotypes, respectively.

Qualitative estimation of Fe and Zn was carried out by rating ranges as high (50.0 mg kg^{-1} or above), medium (37.0-49.0 mg kg^{-1}) and low (<37 mg kg^{-1}) for Fe (Fig. 1) and similarly; high (50 mg kg^{-1} or above), medium (38-49 mg kg^{-1}) and low (<38 mg kg^{-1}) for Zn concentration (Fig. 2). Besides this, reliability of rapid qualitative techniques for iron and zinc assessment was confirmed by standard Atomic Absorption Spectrophotometric technique. Strong positive association of Zn with other elements especially with Fe in the current study was coexisting with some preceding readings on bread wheat (Zhao et al., 2011). Mg associated positively highly significant with Ca whilst Ca positively significantly correlated with Fe. All others exhibited positive association with each other. Due to their positive correlation amongst them, it become imperative that these elements are fundamental for nutrient equilibrium and bioavailability and their correlation should be painstaking. Pandey et al., (2016) also found similar findings while accessing genetic variability for grain nutrients in wheat of diverse regions.

Principal component and cluster analysis

It disjointed total variation into five PCs which supported their utilization for bio-fortification and genetic improvement for bio-fortified wheat. For addressing undernourishment in human being worldwide; White & Broadley (2009) also advocated genetic fortification for these elements in wheat. PC1 have eigen value >1 while PC2 and PC3 were at par to 1 which solicited 69.9% of total variation. The genotypes in PC1 displayed high value of Zn and Cu, PC-3 established high value of Mg and cluster 4 unveiled high values of Fe and Ca. Similarly, PC2 exhibited low content of Cu and cluster 5, validated low content of Fe and Mg (Table 4).

Table 2: Analysis of variance for measured traits in different wheat genotypes

SOV	Df	Means squares				
		Fe	Zn	Ca	Mg	Cu
Treat	49	73016.8 **	44.2928**	3.199 E-04**	1.771 E-04**	23.3047**
Reps	01	75.7	3.1400	5.760 E-06	3.240 E-06	1.1364
Error	-	4.2	1.2249	1.257E-05	9.747 E-07	0.2394
CV	-	0.67	2.56	3.92	0.65	3.64

**=P≤0.01; Fe=Iron; Zn=Zinc; Ca=Calcium; Mg=Magnesium; Cu=Copper

Table 3: Correlation arrangements of minerals

Minerals	Fe	Zn	Ca	Mg	Cu
Fe	1				
Zn	0.163**	1			
Ca	0.079*	0.256**	1		
Mg	0.102	0.157**	0.042**	1	
Cu	0.146	0.298*	0.098	0.103	1

**=P≤0.01; *=P≤0.05

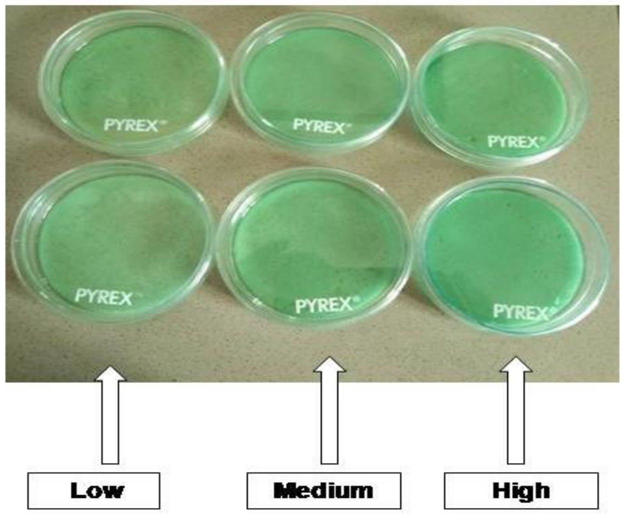

Figure1: High, medium and low iron concentration in wheat grains by Prussian blue procedure

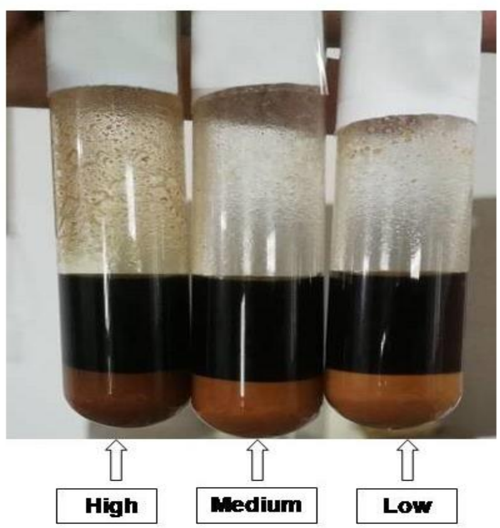

Figure2: High, medium and low zinc concentration in wheat grains by Dithizone Staining Technique

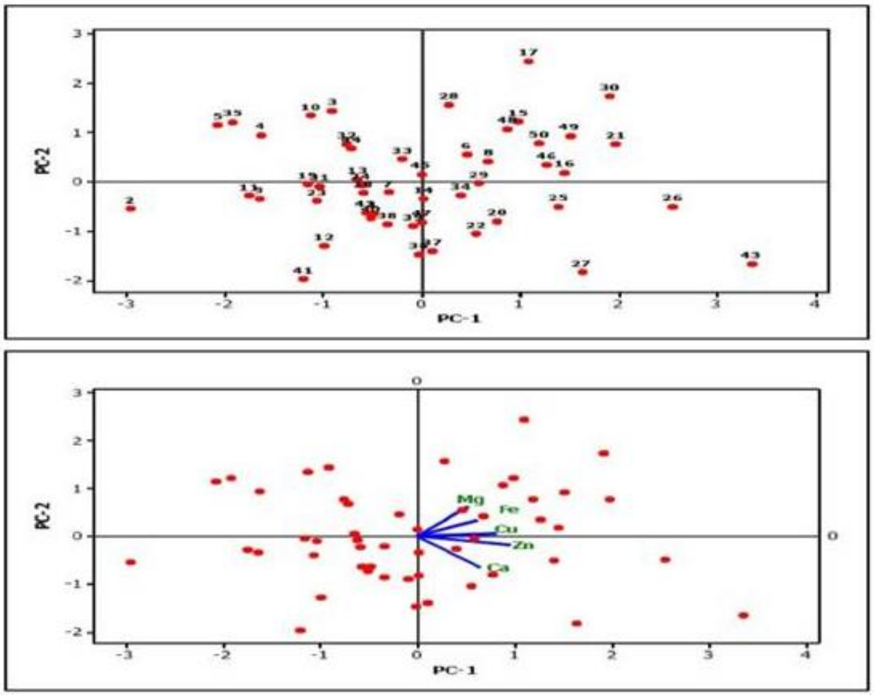

Figure3: Biplot and score plots appearance

Table 4: Principle components analysis of traits studied in different wheat genotypes

Minerals	PC1	PC2	PC3	PC4	PC5
Fe	0.378	0.343	-0.649	0.564	0.031
Zn	0.592	-0.176	0.082	-0.155	-0.767
Ca	0.398	-0.661	0.262	0.415	0.403
Mg	0.317	0.640	0.668	0.159	0.138
Cu	0.497	0.070	-0.240	-0.679	0.479
Eigen values	1.610	0.981	0.903	0.863	0.644
Proportion	0.322	0.196	0.181	0.173	0.129
Cumulative	0.322	0.518	0.699	0.871	1

Table 5: Wheat genotypes connected to different clusters

Cluster No.	Accessions No.	Accessions
Cluster 1	**8**	**1, 7, 14, 20, 22, 29, 28, 35**
Cluster 2	**14**	**2, 9, 11, 19, 23, 31, 32, 33, 44, 45, 3, 4, 5, 10**
Cluster 3	**12**	**12, 41, 36, 37, 47, 38, 40, 39, 13, 18, 24, 42**
Cluster 4	**13**	**34, 6, 8, 50, 46, 25, 15, 21, 17, 16, 26, 27, 43**
Cluster 5	**3**	**30, 48, 49**

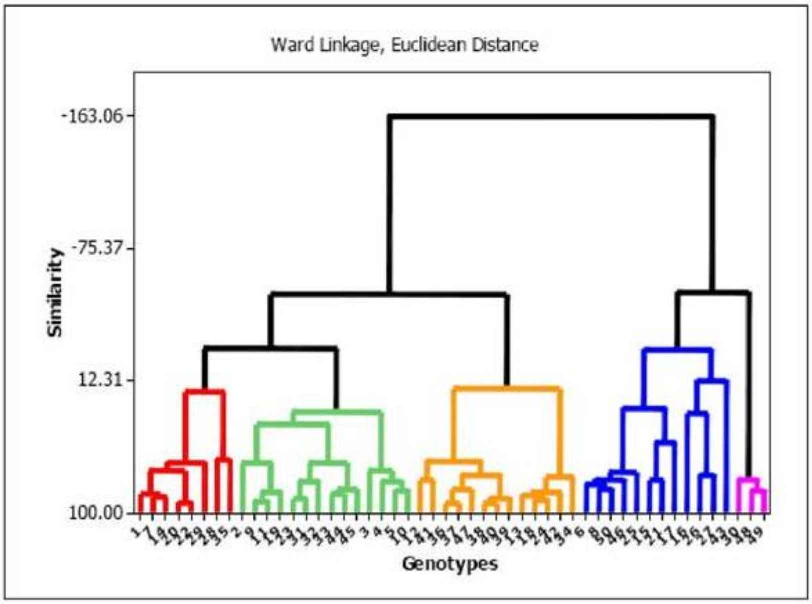

Figure4: Dendrogram showing the linkage of genotypes for dignified traits

The appearance in biplot with their comparative score plot presented the affiliation among traits and genotypes from impulsive and qualitative cataloging of multidimensional data. On biplot, the genotypes those were adjacent together were apparent as being alike when appraised on 5 contents while elements which were more apart were more varied from other genotypes (Fig. 3).

Cluster analysis

The maximum cluster distances from centroid were recorded among cluster 4 (4.4) and cluster 5 (0.87) which showed that these cluster were more genetically diverse from each other than any other clusters (Table 5). The lowest distance was noted between cluster 1 (2.8) and cluster 2 (2.1) which showed that they were fewer genetically classified. The broader distance between the clusters can be employed for evolving transgressive sergeants for the traits thought. The hierarchical alliance showed results comprising of main group each of which was additionally sub distributed into sub-assemblies. The most dissimilar genotypes were 1 and 49. Position interpretation can be made likewise (Fig. 4). Olgunet al., (2018) also grouped genotypes on the basis of similarities for the elements studied via dendrogram.

Quantitative confirmation

The results revealed that all the 50 genotypes grouped themselves in high range for Fe content. Regarding Zn content, high range was found in genotypes 20, 22, 25, 26 and 4, low ranges in genotypes 3, 5, 9 while remaining all other fall under medium range (Abdullah et al., 2018).

CONCLUSION

The present research described the minerals composition and correlation among the essential minerals investigated in bread wheat that is important for appraisal of micronutrient deficiency (particularly iron and zinc) in the counties consuming wheat as staple food. A straightforward correlation uncovered extremely optimistic significant correlation of zinc with all nutrients deliberated in such a way that magnesium linked positively highly significant with calcium that was positively significantly allied with iron. The genotypes in PC-1 exhibited high worth of zinc and copper, PC-3 depicted lofty value of magnesium and cluster 4 explored high values of iron and Ca. The genotypes those were neighboring jointly were obvious as being identical when appraised on five contents while those which were more apart were more diverse from remaining genotypes in biplots. Dithizone staining and Prussian blue techniques were found to be novel, valuable and cost effective techniques for the qualitative assessment of zinc and iron in massive wheat genotypes confirmed by quantitative determination of these minerals by Atomic Absorption Spectrophotometer. All the 50 genotypes knock down in high range (50.0 mg kg^{-1} or above) for iron content but for zinc contents, 5 genotypes fall in high range (50 mg kg^{-1} or above), 3 genotypes in low range (<38 mg kg^{-1}) while all other articulate themselves in medium range (38-49 mg kg^{-1}) during quantitative confirmation. To combat growing malnutrition and rising confront of food security, the genetic stock investigated can be used as a valuable yardstick for wheat crop quality improvement strategy (bio-fortified wheat development) and wheat processing industry.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

All the authors contributed in the manuscript write up. Muhammad Abdullah contributed in conception, design, material preparation, data collection and initial drafting of manuscript. Anjum Javed worked on manuscript design and draft improvement while Muhammad Asghar was involved in data collection and draft improvement. Makhdoom Hussain, Sadaf Shamim, Hira Shair and Aftab Ahmed Khan had contribution regarding conception, design and draft improvement of present study. Majid Nadeem, Skinder Ali Cheema and Amna Kanwal contributed in manuscript conception, design and material preparation while Muhammad Zulkiffal, Nauman Ali and Muhammad Hammad Tanveer worked on conception, design, data collection and draft improvement and, Javed Ahmed and Muhammad Owais extended cooperation regarding conception, design and statistical analysis of the study data. Javed Anwar, Muhammad Zeeshan and Muhammad Yaqub Mujahid had contribution in the field of conception, statistical analysis and draft improvement.

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