https://doi.org/10.29309/TPMJ/2023.30.08.7560

The Professional Medical Journal
www.theprofesional.com
2023, Volume, 30 Issue, 08

ORIGINAL ARTICLE

A comparison of Lipid Profile and Highly Sensitive C Reactive Protein (HsCRP) levels between prehypertensive individuals and those with normal blood pressure.

Rizwan Ali Talpur[1], Yar Muhammad Nizamani[2], Hanozia Hassan[3], Aamir Hussain[4], Ameer Abbas Ali[5], Rabnawaz Sathio[6]

Article Citation: Talpur RA, Nizamani YM, Hassan H, Hussain A, Ali AA, Sathio R. A comparison of Lipid Profile and Highly Sensitive C Reactive Protein (HsCRP) levels between prehypertensive individuals and those with normal blood pressure. Professional Med J 2023; 30(08):1048-1054. https://doi.org/10.29309/TPMJ/2023.30.08.7560

ABSTRACT… Objective: In the current study, hsCRP and lipid profile levels in prehypertensives were assessed and compared with those in normal subjects, as well as blood pressure and lipid profile correlations were examined. **Study Design:** Cross-sectional. **Setting:** Department of Physiology, Liaquat University of Medical & Health Sciences (LUMHS) in Jamshoro. **Period:** Six months 1st November 2022 to 30th April 2023. **Material & Methods:** From the general population, 50 pre-hypertensive and 50 normotensive subjects ranging in age from 25 to 55 were enrolled. A blood pressure measurement and an anthropometric measurement were taken. Student t tests were used to compare serum hsCRP with lipid profiles. In order to correlate blood pressure with hsCRP and lipid profile, Pearson correlation was used. **Results:** A significant correlation was not found between blood pressure (SBP and DBP) and hsCRP in either group. When compared to normal individuals, pre-hypertensives had significantly higher cholesterol and LDL (P-value <0.001). Among prehypertensive patients, blood pressure and lipid parameters did not seem to be significantly correlated. **Conclusions:** We conclude that in both prehypertensive and normal subjects, hsCRP levels are not significantly different. Therefore, pre-hypertensive patients do not have inflammation. Prehypertensive patients have significantly higher total cholesterol and LDL levels than normal individuals. There is an alteration in the lipid profile of prehypertensive individuals.

Key words: Highly Sensitive C Reactive Protein (hsCRP), Lipid Profile, Prehypertension.

INTRODUCTION

Hypertension, commonly known as high blood pressure, is a significant global health concern affecting millions of individuals worldwide.[1] It is a leading risk factor for cardiovascular diseases such as heart attacks and strokes.[2] Prehypertension, a precursor to hypertension, is characterized by blood pressure levels higher than normal but not meeting the criteria for hypertension diagnosis.[3] Both prehypertension and hypertension are associated with an increased risk of cardiovascular events. Lipid profile and highly sensitive C-reactive protein (HsCRP) are important biomarkers used to assess cardiovascular health.[4] Understanding the lipid profile and HsCRP levels in prehypertensive individuals compared to those with normal blood pressure can provide valuable insights into the early stages of cardiovascular disease and may aid in the identification of individuals at a higher risk of developing hypertension and related complications.[5]

Identifying individuals with prehypertension who are at a higher risk of developing hypertension is crucial for implementing preventive measures and interventions to reduce cardiovascular disease burden.[6] Assessing the lipid profile and HsCRP levels in prehypertensive individuals may help in identifying early markers of cardiovascular risk and guide appropriate interventions, such as lifestyle modifications and pharmacotherapy.[7] However, the relationship between prehypertension and lipid profile and HsCRP levels is not fully understood, and there is a gap in the knowledge regarding the specific

1. MBBS, M.Phil (Physiology), Assistant Professor Physiology, Bilawal Medical College for Boys, Liaquat University of Medical & Health Sciences Jamshoro Sindh Pakistan.
2. MBBS, M.Phil (Physiology), Associate Professor Physiology, Isra University Hyderabad, Sindh Pakistan.
3. MBBS, M.Phil (Physiology), Assistant Professor Physiology, Bilawal Medical College, Liaquat University of Medical & Health Sciences Jamshoro, Sindh Pakistan.
4. MBBS, M.Phil (Physiology), Associate Professor Physiology, Bilawal Medical College for Boys at Liaquat University of Medical & Health Sciences Jamshoro, Sindh Pakistan.
5. MBBS, DCP, M.Phil, Associate Professor Pathology, Peoples University of Medical & Health Sciences for Women, Nawabshah -Shaheed Benazirabad, Sindh - Pakistan Nawabshah.
6. MBBS M.Phil (Hematology), Postgraduate Trainee Pathology, Liaquat University of Medical & Health Sciences Jamshoro Sindh Pakistan.

Correspondence Address:
Dr. Rizwan Ali Talpur
Department of Physiology
Bilawal Medical College for Boys,
Liaquat University of Medical &
Health Sciences Jamshoro Sindh, Pakistan.
dr.rizwantalpur@gmail.com
Article received on: 05/05/2023
Accepted for publication: 15/07/2023

differences between prehypertensive individuals and those with normal blood pressure.

We hypothesize that prehypertensive individuals will exhibit alterations in lipid profile and increased levels of highly sensitive C-reactive protein (HsCRP) compared to individuals with normal blood pressure. This research will fill the existing gap in knowledge regarding the relationship between prehypertension and cardiovascular risk factors by focusing on lipid profile and HsCRP levels. While previous studies have investigated the association of lipid abnormalities and inflammation with hypertension, limited research has specifically explored these biomarkers in prehypertensive individuals.[8] The findings of this research can contribute to a better understanding of the cardiovascular risk profile of prehypertensive individuals and may have implications for early intervention and preventive strategies. The purpose of this study is to compare the lipid profile and highly sensitive C-reactive protein (HsCRP) levels between prehypertensive individuals and those with normal blood pressure. By comparing the lipid profile and HsCRP levels between prehypertensive and normotensive individuals, this study will provide valuable insights into the early cardiovascular risk factors associated with prehypertension. The findings will enhance our understanding of the pathological mechanisms underlying prehypertension and contribute to the development of targeted preventive strategies for cardiovascular diseases.

MATERIAL & METHODS

This cross-sectional study was conducted on 100 subjects which was carried out in the department of Physiology, Liaquat University of Medical & Health Sciences (LUMHS) in Jamshoro for six months from 1st November 2022 to 30th April 2023. There were 50 prehypertensive subjects and 50 age and BMI matched normal subjects 25 to 55 years' old who met the inclusion and exclusion criteria. The subjects were equally divided into two groups. Group 1 (n = 50) comprised on Pre-hypertensive while subjects in Group 2 (n = 50) were Healthy normal.

We provided written informed consent to all the subjects. Prehypertensives and normal were recruited through history and clinical examination by students and staff of Liaquat University of Medical & Health Sciences (LUMHS) in Jamshoro.

Inclusion Criteria

1. Participants aged 18 years or older.
2. Both males and females.
3. Individuals with prehypertension (defined as systolic blood pressure ranging from 120-139 mmHg and/or diastolic blood pressure ranging from 80-89 mmHg).
4. Individuals with normal blood pressure (defined as systolic blood pressure <120 mmHg and diastolic blood pressure <80 mmHg).
5. Availability of complete lipid profile and highly sensitive C-reactive protein (hsCRP) data.
6. Participants who have provided informed consent to participate in the study.

Exclusion Criteria

1. Participants with a history of diagnosed hypertension or antihypertensive medication use, history of cardiovascular diseases (e.g., myocardial infarction, stroke, congestive heart failure).
2. Participants with a history of diabetes mellitus, chronic kidney disease, Pregnant women or women who are breastfeeding, known inflammatory conditions (e.g., rheumatoid arthritis, lupus), liver diseases (e.g., cirrhosis, hepatitis), known endocrine disorders (e.g., thyroid dysfunction, Cushing's syndrome), malignancies.

Ethical clearance was taken from the Ethical Committee of Liaquat University of Medical & Health Sciences (LUMHS) in Jamshoro by letter Number NO. LUMHS/REC/830/10/2022.

The participants were screened for general physical health. WHO guidelines were followed when measuring and interpreting anthropometric parameters.

Height

A vertical board with a metric ruler was measured against it, and a horizontal headboard was

placed against the uppermost point. During the recording, the subject stood barefoot on a flat surface. Weight was evenly dispersed between their feet and heels. The head was located perpendicular to the body for a perpendicular line of vision. There was a vertical board in contact with the head, back, buttocks, and heels, as well as arms hanging freely at the sides. A full erect posture was required while the individual inhaled deeply. In order to estimate height to the nearest 0.1 cm, we measured the uppermost point on the head that produces enough pressure on the hair to bandage it.

Weight
Weighing was conducted without footwear and wearing light clothing, standing straight on the center of the machine with both feet evenly distributed by an ISI certified weighing machine to the nearest 100 grams.

Body Mass Index (Quetelet's index) Calculated as wt (kg)/ht2 (mt2)

Waist Circumference
In the horizontal plane, the measurement was taken halfway among the inferior margin of the last rib and the crest of the ilium. To the nearest 0.1 cm, it was taken at the end of expiration. Soft tissues were not compressed by the tape. Sphygmomanometers were used to measure blood pressure in the right arm of seated participants following 5 minutes of rest. The 1st and 5th Korotkoff sounds were used to record it to the nearest 2 mmHg. SBP was determined based on the appearance of the 1st Korotkoff sound. DBP was calculated based on the 5th Korotkoff sound. Analysis was performed using the mean of the last two blood pressure measurements.

After a 12-hour overnight fast, a blood sample of 5 ml was collected from the antecubital vein using vacutainers and disposable needles, after the necessary precautions had been taken. Immediately after collecting the samples, they were left undisturbed for half an hour to allow the clots to form completely. Following centrifugation, the serum was separated from the clot. To conduct the analysis, the serum was stored at-200 C in Eppendorf tubes after centrifugation.

Statistical Analysis
A descriptive statistical analysis was conducted in the present study. In continuous measurements, Mean ± SD (Min - Max) is used, while in categorical measurements, Number (%) is used. A p-value of 0.05 is considered to be significant. Graphs, tables, etc. Were generated using statistical programs such as SPSS 23.0, Microsoft Word, and Excel.

RESULTS

A total of 100 patients were selected in this study. Fifty participants with prehypertension and 50 patients with normal subjects. In order to determine whether there was a significant variance among the two groups in terms of age, height, weight, BMI, and waist circumference, we used the two-tailed independent t-test. It was not seen that there were any significant changes among the two groups when comparing basic characteristics such as age, height, weight, BMI, and waist circumference (P-value >0.05). The basic characteristics of both groups were similar. Based on comparisons of blood pressure values among the two groups, we found a significant change (P-value <0.0001).

Fasting blood sugar levels and hsCRP levels were compared between the two groups. In this comparison, there was no statistically significant change (P-value 0.237).

Lipid parameters compared between the two groups. TC (P-value < 0.0001) and LDL (P-value < 0.001) values were significantly higher in prehypertensives than normals. In regards to triglycerides (P-value 0.775), HDL (P-value 0.442), and VLDL (P-value 0.775), statistically significant changes were not perceived among the groups.

These results suggest that there are differences in blood pressure, fasting blood sugar, and lipid parameters between pre-hypertensive and normal individuals, which may be relevant for further health assessments and interventions. (Table-I)

The relationship between serum hsCRP levels and blood pressure are shown in Table-II. There is a weak correlation between hsCRP and both SBP and DBP, with a correlation coefficient of -0.045 (P-value of 0.726) and 0.128 (P-value of 0.273) for pre-hypertensive individuals, and -0.036 (P-value of 0.673) and -0.012 (P-value of 0.074) for normal individuals, respectively.

The table also shows that there is a moderate to strong correlation between age and lipid parameters, with higher age being positively correlated with total cholesterol, triglycerides, and VLDL and negatively correlated with HDL. The strongest correlation is seen between age and total cholesterol, with a correlation coefficient of 0.481 (P-value < 0.0001) for pre-hypertensive individuals and 0.347 (P-value 0.215) for normal individuals.

The correlation between SBP and lipid parameters is weak and statistically not significant, except for the negative correlation between SBP and HDL for pre-hypertensive individuals (correlation coefficient of -0.266, P-value of 0.025).

The correlation between DBP and lipid parameters is also weak and statistically not significant, except for the negative correlation between DBP and HDL for normal individuals (correlation coefficient of -0.318, P-value of 0.005)

DISCUSSION

Prehypertensive and normal subjects were compared with regards to serum hsCRP and serum lipid profile, and these parameters were linked with blood pressure. According to the present research, there was no statistically significant change in serum hsCRP levels among pre-hypertensive and normal controls (P-value 0.237). There is no inflammation in prehypertensive subjects. Results similar to the present study were shown by Eun Hee Nah et al.[9] Prehypertensives have a higher level of hsCRP than normal, according to a few studies.[10,11]

Among the two groups in the present study, hsCRP levels did not differ significantly. According to the current research, the results of this study could be accounted for by the age group of the study participants, who were on average 28.24±5.36 years for cases and 29.42±5.76 years for controls. According to the studies, prehypertensives have higher levels of hsCRP than normal subjects whose age group is older. There are no independent associations between inflammation and hsCRP levels and prehypertension.[12,13]

Demographic Variables	Pre-hypertensive	Normal	P-Value
Age in years	28.24±5.36	29.42±5.76	0.221
Weight (kg)	68.89±12.13	68.02±11.33	0.572
Height (cm)	173.41±7.42	171.12±7.72	0.175
BMI (kg/m^2)	23.78±3.05	24.13±2.89	0.576
Waist Circumference (cm)	78.87±8.87	80.31±9.86	0.425
Comparison of blood pressure			
SBP (mm Hg)	132.89±4.87	115.31±5.53	<0.0001*
DBP (mm Hg)	84.89±3.42	73.81±5.87	<0.0001*
Comparison of fasting blood sugar			
FBS (mg/dl)	86.14±9.21	82.03±9.71	0.046*
Comparison of hsCRP			
hsCRP levels (mg/L)	1.51±0.53	1.22±0.18	0.237
Comparison of lipid parameters			
Triglycerides (mg/dl)	100.42±42.43	99.24±33.08	0.775
Total cholesterol (mg/dl)	167.12±37.14	130.87±22.78	<0.0001*
HDL (mg/dl)	55.34±13.86	53.25±14.79	0.442
VLDL(mg/dl)	20.09±8.37	19.87±6.54	0.775
LDL(mg/dl)	92.87±36.38	58.65±25.54	<0.001*

Table-I. The comparison of pre-hypertensive and normal demographic variables.

Pair	Pre-hypertensive		Normal	
	r value	P-Value	r value	P-Value
hsCRP vs SBP (mm Hg)	-0.045	0.726	-0.036	0.673
hsCRP vs DBP (mm Hg)	0.128	0.273	-0.012	0.074
Correlation of blood pressure and age with lipid parameters (mg/dl)				
Age in years vs Total cholesterol	0.481	<0.001*	0.347	0.215
Age in years vs Triglycerides	0.452	<0.001*	0.214	0.031*
Age in years vs HDL	-0.015	0.824	0.214	0.231
Age in years vs LDL	0.266	0.015*	-0.001	0.768
Age in years vs VLDL	0.472	<0.001*	0.316	0.032*
Systolic blood pressure (mm Hg)				
SBP vs Total cholesterol	0.046*	0.615	0.036	0.747
SBP vs Triglycerides	0.041*	0.543	0.066	0.527
SBP vs LDL	0.062	0.588	0.214	0.134
SBP vs HDL	-0.042	0.681	-0.266	0.025*
SBP vs VLDL	0.051	0.641	0.066	0.532
Diastolic blood pressure (mm Hg)				
DBP vs Total cholesterol	0.127	0.133	0.017	0.813
DBP vs Triglycerides	-0.035	0.713	-0.017	0.712
DBP vs HDL	0.131	0.446	-0.318	0.005*
DBP vs LDL	0.203	0.221	0.271	0.067
DBP vs VLDL	-0.035	0.723	-0.017	0.811

Table-II. The relationship between hsCRP levels and blood pressure.

This could be an age-related phenomenon. Furthermore, a rise in hsCRP can be a consequence of prehypertension rather than its cause. A significant change among participants with hypertension and normal subjects was observed in total cholesterol ($p<0.001$) and LDL ($p<0.001$). When compared to normals, prehypertensives have a higher risk of cardiovascular events. Epidemiological studies have revealed similar findings.[14,15] Accordingly, prehypertensives have significantly elevated lipid parameters compared to normals, while their hsCRP levels are not much different. This proposes that lipid parameters are raised in prehypertensives much earlier, even before inflammation begins. According to studies done in America, prehypertension is also related with risk factors such as hypercholesterolemia, diabetes, and obesity.[16]

There is a higher prevalence of metabolic syndrome among prehypertensives compared to normals based on studies conducted in Korean populations.[17] According to an Israeli study, prehypertensives have a higher ten-year risk of cardiovascular disease than normal people due to hyperglycemia, dyslipidemia, obesity and metabolic syndrome.[14] The study found that prehypertensives also had elevated levels of cholesterol and LDL. JNC 7 published in 2003 identified these individuals as potential candidates for cardiovascular interventions and risk reduction. Before the publication of JNC 7, their blood pressure was considered "normal." However, we now know that prehypertension rises the risk of cardiovascular events. The risk of progression to hypertension is also high among prehypertensives. Studies have begun to examine the role of pharmacological interference in prehypertensives at risk of cardiovascular events.[18]A study found that prehypertension alone accounts for 9.1% of deaths among Americans aged 25-74 years old, 6.5% of nursing home admissions, and 3.4% of hospitalizations.[19] lifestyle modification or pharmacological intervention is required based on these results.

CONCLUSION

Total cholesterol and LDL levels are significantly high in prehypertensives as compared to normals. In both prehypertensives and normals, SBP and DBP are not significantly correlated with hsCRP levels.

REFERENCES

1. Mills KT, Stefanescu A, He J. **The global epidemiology of hypertension.** Nat Rev Nephrol [Internet]. 2020 Apr 5; 16(4):223-37. Available from: http://www.nature.com/articles/s41581-019-0244-2

2. Kjeldsen SE. **Hypertension and cardiovascular risk: General aspects.** Pharmacol Res [Internet]. 2018 Mar; 129:95-9. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1043661817311180

3. Svetkey LP. **Management of Prehypertension.** Hypertension [Internet]. 2005 Jun; 45(6):1056-61. Available from: https://www.ahajournals.org/doi/10.1161/01.HYP.0000167152.98618.4b

4. Kamath D, Xavier D, Sigamani A, Pais P. **High sensitivity C-reactive protein (hsCRP) & amp; cardiovascular disease: An Indian perspective.** Indian J Med Res [Internet]. 2015; 142(3):261. Available from: http://www.ijmr.org.in/text.asp?2015/142/3/261/166582

5. Chobanian A V., Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL, et al. **Seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure.** Hypertension [Internet]. 2003 Dec; 42(6):1206-52. Available from: https://www.ahajournals.org/doi/10.1161/01.HYP.0000107251.49515.c2

6. AlKaabi LA, Ahmed LS, Al Attiyah MF, Abdel-Rahman ME. **Predicting hypertension using machine learning: Findings from Qatar Biobank Study.** Shimosawa T, editor. PLoS One [Internet]. 2020 Oct 16; 15(10):e0240370. Available from: https://dx.plos.org/10.1371/journal.pone.0240370

7. Arnett DK, Blumenthal RS, Albert MA, Buroker AB, Goldberger ZD, Hahn EJ, et al. **2019 ACC/AHA Guideline on the Primary Prevention of Cardiovascular Disease: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines.** Circulation [Internet]. 2019 Sep 10;140(11). Available from: https://www.ahajournals.org/doi/10.1161/CIR.0000000000000678

8. Ghazi L, Baker J V, Sharma S, Jain MK, Palfreeman A, Necsoi C, et al. **Role of inflammatory biomarkers in the prevalence and incidence of hypertension among HIV-Positive participants in the START Trial.** Am J Hypertens [Internet]. 2020 Jan 1; 33(1):43-52. Available from: https://academic.oup.com/ajh/article/33/1/43/5564372

9. Nah EH, Kim HC. **Comparison of cardiovascular risk factors between normotension and prehypertension.** Ann Lab Med [Internet]. 2007 Oct 1;27(5):377–81. Available from: http://annlabmed.org/journal/view.html?doi=10.3343/kjlm.2007.27.5.377

10. Sathiyapriya V, Selvaraj N, Nandeesha H, Bobby Z, Aparna A, Pavithran P. **Association between protein bound sialic acid and high sensitivity C-Reactive protein in prehypertension: A possible indication of underlying cardiovascular risk.** Clin Exp Hypertens [Internet]. 2008 Jan 3; 30(5):367-74. Available from: http://www.tandfonline.com/doi/full/10.1080/10641960802275106

11. Gupta AK, Johnson WD. **Prediabetes and prehypertension in disease free obese adults correlate with an exacerbated systemic proinflammatory milieu.** J Inflamm [Internet]. 2010; 7(1):36. Available from: http://journal-inflammation.biomedcentral.com/articles/10.1186/1476-9255-7-36

12. Erren M, Reinecke H, Junker R, Fobker M, Schulte H, Schurek JO, et al. **Systemic inflammatory parameters in patients with atherosclerosis of the coronary and peripheral arteries.** Arterioscler Thromb Vasc Biol [Internet]. 1999 Oct; 19(10):2355-63. Available from: https://www.ahajournals.org/doi/10.1161/01.ATV.19.10.2355

13. Cao JJ, Thach C, Manolio TA, Psaty BM, Kuller LH, Chaves PHM, et al. **C-Reactive Protein, Carotid Intima-Media Thickness, and Incidence of Ischemic Stroke in the Elderly.** Circulation [Internet]. 2003 Jul 15; 108(2):166-70. Available from: https://www.ahajournals.org/doi/10.1161/01.CIR.0000079160.07364.6A

14. Grotto I, Grossman E, Huerta M, Sharabi Y. **Prevalence of prehypertension and associated cardiovascular risk profiles among young israeli adults.** Hypertension [Internet]. 2006 Aug; 48(2):254-9. Available from: https://www.ahajournals.org/doi/10.1161/01.HYP.0000227507.69230.fc

15. Pletcher MJ, Bibbins-Domingo K, Lewis CE, Wei GS, Sidney S, Carr JJ, et al. **Prehypertension during Young Adulthood and Coronary Calcium Later in Life.** Ann Intern Med [Internet]. 2008 Jul 15; 149(2):91. Available from: http://annals.org/article.aspx?doi=10.7326/0003-4819-149-2-200807150-00005

16. Greenlund KJ, Croft JB, Mensah GA. **Prevalence of heart disease and stroke risk factors in persons with prehypertension in the United States, 1999-2000.** Arch Intern Med [Internet]. 2004 Oct 25; 164(19):2113. Available from: http://archinte.jamanetwork.com/article.aspx?doi=10.1001/archinte.164.19.2113

17. Choi KM, Park HS, Han JH, Lee JS, Lee J, Ryu OH, et al. **Prevalence of prehypertension and hypertension in a Korean population: Korean National Health and Nutrition Survey 2001.** J Hypertens [Internet]. 2006 Aug; 24(8):1515-21. Available from: https://journals.lww.com/00004872-200608000-00010

18. Julius S, Nesbitt SD, Egan BM, Weber MA, Michelson EL, Kaciroti N, et al. **Feasibility of treating prehypertension with an angiotensin-receptor blocker.** N Engl J Med [Internet]. 2006 Apr 20; 354(16):1685-97. Available from: http://www.nejm.org/doi/abs/10.1056/NEJMoa060838

19. Russell LB, Valiyeva E, Carson JL. **Effects of prehypertension on admissions and deaths.** Arch Intern Med [Internet]. 2004 Oct 25; 164(19):2119. Available from: http://archinte.jamanetwork.com/article.aspx?doi=10.1001/archinte.164.19.2119.

AUTHORSHIP AND CONTRIBUTION DECLARATION

No.	Author(s) Full Name	Contribution to the paper	Author(s) Signature
1	Rizwan Ali Talpur	Designed the research, assessed the vases, wrote the paper, Interpretation of discussion and data entry in SPSS.	
2	Yar Muhammad Nizamani	Collected the data, did the literature search, drafted the manuscript assisted in writing the paper.	
3	Hanozia Hassan	Involved in data collection, analyzed the data revised the manuscript, Proof reading, Help in methodology.	
4	Aamir Hussain	Revised the original manuscript, Reviewed the cases, analyzed the data and assisted in writing the paper, Interpretation inm results writing.	
5	Ameer Abbas Ali	References, citation manager & amp, designing of results and charts and Graphs in manuscript.	
6	Rabnawaz Sathio	Data Entry in SPSS and other technical help, help in corrections.