CORRELATION OF INFLAMMATORY MARKERS WITH TYPE 2 DIABETES MELLITUS IN PAKISTANI PATIENTS

Amina Nadeem[1], Abdul Khaliq Naveed[2], Muhammad Mazhar Hussain[3], Syed Irfan Raza[4]

ABSTRACT

Objective: *To study the levels of inflammatory markers in Type 2 Diabetes Mellitus Pakistani patients and determine their correlation with the disease.*

Methodology: *This cross sectional study was conducted at Military Hospital, Rawalpindi, and Centre for Research in Experimental and Applied Medicine (CREAM), Army Medical College, Rawalpindi, Pakistan from June 2011-June 2012. We determined the levels of Erythrocyte Sedimentation Rate (ESR), Total Leukocyte Count (TLC), Interleukin 6 (IL-6) and Tumor Necrosis Factor- alpha (TNF-α) in 72 patients with Type 2 Diabetes Mellitus (T2DM) and 40 healthy controls. The levels were correlated with age, duration of the disease, Body Mass Index (BMI), lipid profile and insulin resistance.*

Results: *ESR, TLC, IL-6 and TNF-α were found to be significantly higher in T2DM patients. There was also statistically significant difference in BMI, fasting blood sugar, insulin resistance and dyslipidemia (except LDL) between diabetic and control group. All inflammatory markers except TNF-α were positively correlated with insulin resistance. Only TNF-α was found to be correlated to duration of the disease. Both TLC and Serum insulin were positively correlated to BMI. Inflammatory markers were not correlated to dyslipidemia.*

Conclusion: *This is the first study reporting levels of cytokines in Pakistani diabetic patients and healthy controls. There is augmented inflammation in T2DM in Pakistani patients which plays role in higher insulin resistance in these patients. TNF-α levels increases with longer duration of the disease. Levels of inflammatory markers are not correlated to BMI, dyslipidemia or increasing age.*

Key Words: *Diabetes Mellitus, Inflammation, Interleukin-6 (IL-6), Tumor necrosis factor-alpha (TNF-α).*

This article may be cited as: Nadeem A, Naveed AK, Hussain MM, Raza SI. Correlation of inflammatory markers with type 2 Diabetes Mellitus in Pakistani patients. J Postgrad Med Inst 2013; 27(3):267-73.

INTRODUCTION

The prevalence of diabetes mellitus (DM) worldwide was estimated to be 171 million people in the year 2000 and this is projected to increase to 366 million by the year 2030[1]. In Pakistan, there were 5.2 million people with diabetes in the year 2000 and is estimated to increase to about 13.9 million by the year 2030, making Pakistan; the country with the fourth highest number of diabetics in the world[2]. Inflammation plays important role in pathogenesis of the disease and inflammatory markers including interleukins, tumor necrosis factor-alpha (TNF- α), C- reactive protein (CRP), tissue plasminogen activator (tPA), heptoglobulin and fibrinogen levels are found to be increased in type 2 DM (T2DM) patients[3]. Adipocytes secrete inflammatory cytokine, in addition they develop macrophage infiltration (adiposities) which is the source of almost all TNF–α and most of the IL-6 in adipose tissue along with other inflammatory markers[4]. Not only obesity but hyperinsulinism per se, seen in metabolic syndrome and type 2 DM, can induce rise in inflammatory markers including IL-6, TNF-α and CRP[3]. High levels of IL-6 and TNF-α have been reported to be

[1,3] Department of Physiology, Army Medical College, National University of Sciences and Technology, Islamabad, Pakistan

[2,4] Department of Biochemistry, Army Medical College, National University of Sciences and Technology, Islamabad, Pakistan

Address for Correspondence:
Dr. Amina Nadeem,
Assistant Professor of Physiology,
Army Medical College, National University of Sciences and Technology, Islamabad - Pakistan.
E-mail: aminanadeem@amcollege.nust.edu.pk

Date Received: October 23, 2012
Date Revised: May 02, 2013
Date Accepted: May 14, 2013

associated with insulin resistance in adipocytes, hepatocytes and myocytes[5-7]. Raised serum IL-6 and TNF-α level in type 2 diabetic patients were found to be associated with increased BMI, fasting insulin levels and insulin resistance[8]. In a prospective study in American females, the baseline CRP and IL-6 levels were significantly higher in those who later developed T2DM[9].

Few studies have been done in Pakistani T2DM patients for estimation of leukocyte count and CRP. No study so far has been done on serum levels of IL-6 and TNF- α level in T2DM patients. The present study was done to evaluate not only the serum levels but also the association of the inflammatory markers with age, duration of the disease, insulin resistance and BMI and lipid profile.

METHODOLOGY

This cross sectional study was conducted at Military Hospital, Rawalpindi, and Centre for Research in Experimental and Applied Medicine (CREAM), Army Medical College, Rawalpindi, National University of Science and Technology (NUST), Pakistan from June 2011-June 2012. Approval from ethical committee of Army Medical College, National University of Sciences and Technology (NUST) was obtained before undertaking the research project. The study was conducted in accordance with the current Good Clinical Practices and the Declaration of Helsinki.

A total of 112 male and female subjects; age being 30-50 years were included in the study. They were divided into study and control groups. The study group (n = 72) were diagnosed cases of type 2 diabetes mellitus of maximum duration of 5 years. The control group (n = 40) comprised of normal healthy individuals. The subjects were selected in accordance with inclusion and exclusion criteria. The inclusion criteria comprised of T2DM patients diagnosed according to 1999 WHO Diabetes criteria. Smokers, type 1 diabetic patients, patients on insulin therapy, patients with diabetic complications and patients with acute or chronic inflammatory disease were excluded from the study.

All study patients had proper history, physical examination and laboratory investigations including blood complete picture, erythrocyte sedimentation rate after one hour by Westergren's method. All subjects were informed about the nature, significance, implications and consequences of the study. They were appraised regarding investigational procedures. They were advised not to do any exercise or take any non-steroidal anti inflammatory drugs (NSAIDs) for two weeks prior to the blood sampling. The written informed consent was obtained and personal particulars of each subject were noted and each was evaluated on the basis of detailed medical history, physical examination and laboratory tests.

10 ml of venous blood sample was drawn from antecubital vein under aseptic conditions in a disposable syringe from each individual. Blood was allowed to clot for 30 minutes at room temperature. After retraction of the clot, serum was separated by centrifugation at 3000 cycles per second for 15 minutes. Serum was then transferred to small sterile tubes and stored at - 20 0C prior to biochemical analysis. Blood CP was performed on sysmex automated analyzer, ESR was determined by Westergren's method, Total cholesterol (TC) by immune-inhibition method, triglycerides (TGs) by calorimetric method, low density lipoprotein (LDL) by immune-inhibition method, high density lipoprotein (HDL); calculated by Freidewald formula and IL-6 and TNF-α levels were determined by Enzyme Amplified Sensitivity Immunoassay (EASIA). Insulin resistance was calculated by Homeostatic Model of Assessment of insulin resistance (HOMA-IR)[10]. Statistical analysis was done on SPSS-17.

IL-6 assay

Quantitative measurement of IL-6 was done by use of a commercial EASIA kit; 'Bioscience Human IL-6 EASIA kit' (Catalogue no KAC1751, BioSource Europe S.A. Rue de I'Industrie 8 B-1400 Nivelles Belgium). The assay is a solid phase Enzyme Amplified Sensitivity Immunoassay performed on microtiter plate. The minimum detectable concentration was estimated to be 2 pg/ml. The intra-assay and inter-assay coefficient of variation for IL-6 were 5.6% and 7.5% respectively.

TNF-*α* assay

Quantitative measurement of TNF- α was done by 'Bioscience Human IL-6 EASIA kit' (Catalogue no KAC1261, BioSource Europe S.A. Rue de I'Industrie 8 B-1400 Nivelles Belgium). The minimum detectable concentration was estimated to be 3pg/ml. The intra-assay and inter-assay coefficient of variation for TNF- α were 5.2 % and 8.0 % respectively.

Statistical analysis was done on SPSS -17. Mean ± SD of age of subjects, duration of the disease, ESR, TLC serum IL-6 and TNF- α levels was determined in both study and control groups. Independent sample t test was applied to compare age, duration of the disease, ESR, TLC, S.IL-6 and S. TNF- α levels in study and control groups. Pearson's coefficient of correlation was done and regression coefficient (R value) determined to assess the association of in-

flammatory markers; ESR, TLC, S.IL-6 and S. TNF-α levels with age, duration of the disease, insulin resistance, BMI and lipid profile. P value < 0.05 was considered statistically significant.

RESULTS

A total of 112 subjects were included in the study. Among them, 72 (40 males; 55.5% and 32 females; 45.5%) were diagnosed cases of T2DM and 40 (27 males and 13 females) were healthy controls. Among 72 T2DM patients 46 (63.8 %), were obese and 26 (36.2%) were non-obese based on BMI. TC, TGs and HDL were significantly higher in T2DM patients than healthy controls, whereas difference in LDL levels was not significantly different in both groups. Serum blood sugar (F) and insulin resistance were significantly higher in diabetics (p value = 0.0001 and 0.001) while Serum insulin was not (p value = 0.81). The inflammatory markers studied were ESR, TLC, IL-6 and TNF-α. The serum levels of all inflammatory markers except ESR are significantly higher in T2DM patients as compared to healthy controls. The mean values ± SD range and p value of demographic characteristics and all biochemical tests in study and control groups (Table 1).

In T2DM patients, age did not show any correlation with any of the inflammatory markers on regression analysis. TNF-α showed very strong positive correlation with duration of the disease (p value = 0.01, R coefficient = 2.7) whereas Serum insulin showed strong negative correlation with duration of the disease on regression analysis (p value= 0.03, R coefficient = - 1.1). Serum IL -6, ESR and TLC was not found to be correlated to duration of the disease. There was weak positive correlation between ESR and BMI (p value= 0.07, R coefficient = 0.43) as well as between Serum insulin and BMI (p value= 0.04, R coefficient = 0.4). Insulin resistance showed weak positive correlation with ESR (p value= 0.009, R coefficient = 0.09), TLC (p value= 0.009, R coefficient = 0.4) and IL-6 (p value= 0.023, R coefficient = 0.16). as shown in figure 1 and 2. Serum Insulin showed strong positive correlation with insulin resistance (p value = 0.0001, R coefficient = 2.34) as shown in figure 3. In lipid profile, none of the inflammatory marker showed any correlation with serum lipid profile except for weak correlation of TLC with S. TG (p value = 0.04, R coefficient=0.3).

Table 1: levels of biochemical parameters in type 2 diabetes mellitus patients and healthy controls

Parameter	Study		Control		p value
	(Mean ± SD)	Range	(Mean ± SD)	Range	
Age in years	46.8 ± 4.09	30 - 50	36.4 ± 6.94	28 - 50	0.06
BMI (kg/m^2)	27.29 ± 4.12	19.2-38.4	24.78 ± 5.16	15.6-35.6	0.006
S. Cholesterol (mmol/L)	4.71 ± 0.96	2.94 - 6.77	4.34 ± 0.93	2.22-6.27	0.05
S. TGs (mmol/L)	2.26 ± 1.16	0.78 - 5.94	1.68 ± 1.02	0.49 – 5.34	0.008
S. LDL (mmol/L)	2.6 ± 0.74	0.6 - 4.62	2.7 ± 0.7	1.1 – 4.24	0.47
S. HDL (mmol/L)	1.1 ± 0.25	0.49 - 2.34	1.02 ± 0.21	0.75 – 1.65	0.05
Fasting Blood Sugar (mmol/L)	8.2 ± 1.9	3.4-13.3	5.2 ± 0.58	4 - 6.5	0.0001
ESR mm (after 1 hour)	23.62 ± 8.62	8 - 45	19.95 ± 9.27	7 - 42	0.03
TLC (n x 10^9)	7.35 ± 1.69	4.1 - 14.1	6.35 ± 1.24	6.4 - 10.1	0.002
IL-6 (pg/ml)	7.25 ± 4.4	0.28 - 21.93	4.7 ± 2.1	0.43 - 7.92	0.001
TNF-α (pg/ml)	28.75 ± 14.19	8 - 85.3	17.09 ± 7.92	8.8 - 34.38	0.0001
S. Insulin (F) mIU/L	13.2 ± 6.9	4.9 - 43.1	13.4 ± 5.12	7.1 - 26.9	0.81
HOMA-IR	4.78 ± 2.61	1.45 – 18.8	3.14 ± 1.21	1.58 – 6.28	0.001

Figure 1: Correlation of TLC with insulin resistance in type 2 diabetic patients

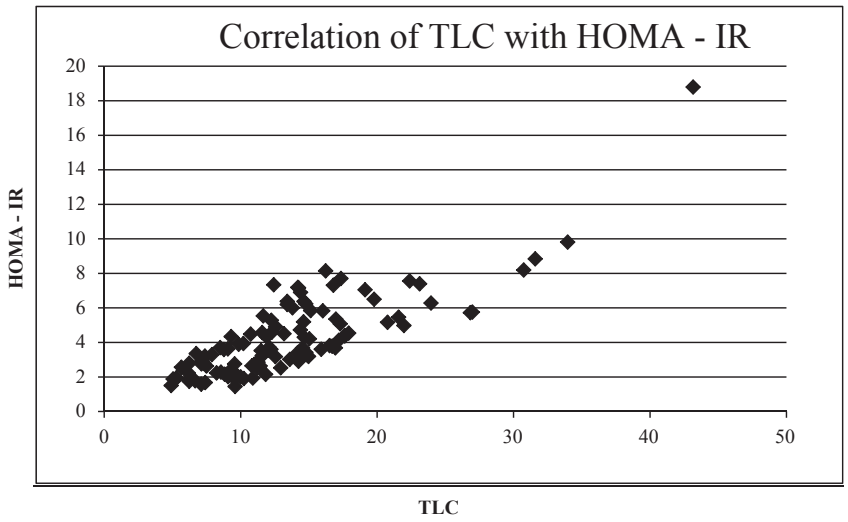


Figure 2: Correlation of IL-6 with insulin resistance in type 2 diabetic patients

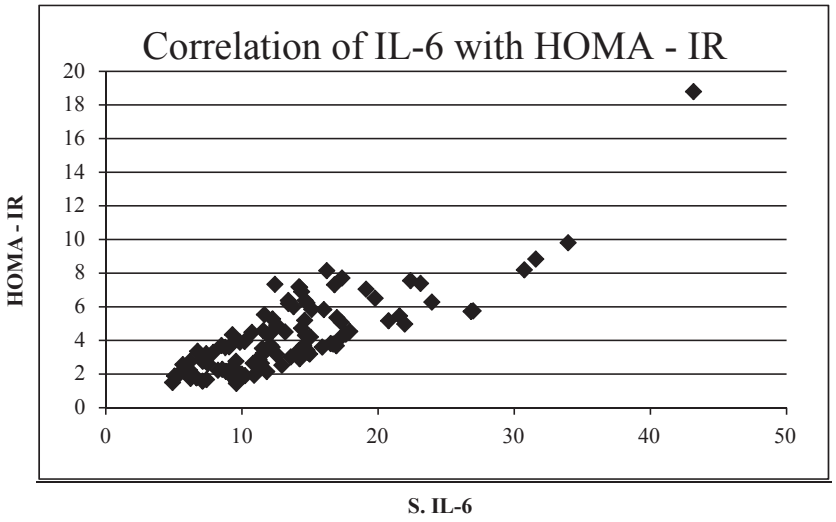
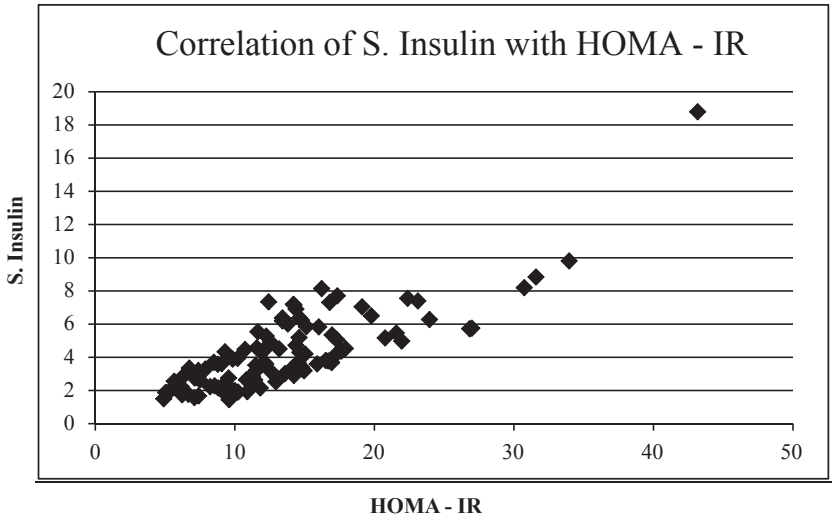


Figure 3: Correlation of S. Insulin levels with insulin resistance in type 2 diabetic patients



DISCUSSION

Subclinical inflammation and presence of almost all indicators of systemic inflammation are found in type 2 diabetic patients. The rise in the inflammatory cytokines is the essential step in glucotoxicity and lipotoxicity induced mitochondrial injury, oxidative stress and beta cell apoptosis in type 2 DM[11]. No study so far has reported the role of cytokines in T2DM in Pakistani population.

Our study found higher levels of ESR, TLC, IL-6 and TNF-α in T2DM patients than in healthy controls as reported in other studies[5-7, 12] indicating the role of inflammation in pathogenesis of the disease. Inflammatory markers were not found to be higher with increasing age. None of the inflammatory markers were found to be correlated with duration of the disease except TNF-α which showed strong positive correlation. ESR and Serum insulin showed weak positive correlation with BMI as also reported in the international studies[8, 13, 14]. It is known already that the source of almost all of IL-6 and most of TNF-α is adipose tissue[7]. However, in our study, the serum levels of IL-6 and TNF- α did not show correlation with BMI. Probably the relationship between cytokine levels and BMI is not linear. The presence of abdominal obesity rather than higher BMI is definitely associated with augmented insulin resistance and indirectly higher cytokines levels as well. This could be possible explanation for absence of correlation of inflammatory markers with BMI and possibly for lack of correlation with dyslipidemia as well in our study. All Inflammatory markers were found to be positively correlated to insulin resistance indicating the role of inflammation in pathogenesis of insulin resistance as already reported in literature.

As far as mean values of serum levels of inflammatory markers are concerned, these were found to be very high in obese diabetic patients in a recent study on Indian population[15] but in our study the mean values of S. IL-6 and TNF- α levels were less than those reported in Indian population. Ruge et al used hyperinsulinemic euglycemic clamp and there was 4 fold increase in IL-6 in type 2 DM patients against 2.5 fold increase in controls. Unlike our findings, there was no significant increase in TNF-α[16]. In a study in US citizens[17], S. IL-6 was found to be higher in T2DM as in our study though levels were less than those in our subjects (4.16 $\pm$ 1.18 vs 7.25 $\pm$ 4.4 pg/ml). Qi et al study results about S. IL-6 levels in T2DM patients are comparable to our study but their serum levels were 2.28 $\pm$ 1.19 pg/ml[18]. Mean values of IL-6 and TNF-α are found to be lower in international studies done in Caucasians as compared to our study. Conversely, our serum values are lower than those reported in Indian population. Limited studies are done in South Asian population and our study is the first one reporting cytokines levels in diabetic patients.

IL-6 -174 G/C Single nucleotide polymorphisms (SNP) and TNF- α -308G/A SNP play role in increased transcription and higher levels of these cytokines[19, 20]. No study has been done so far to determine the role of these SNPs in Pakistani population which can influence the serum levels of these cytokines and ultimately increased insulin resistance.

Regarding association of non-specific inflammatory markers with type 2 DM, very few studies are done in Pakistan. Total Leukocyte Count was found to be significantly increased in diabetic patients that is in accordance to our study[21, 22]. In Pakistan, though serum levels of IL-6 and TNF-α are not reported in diabetic patients but higher S.TNF-α and S. IL-6 levels are reported in coronary artery disease patients as compared to healthy controls[23, 24]. In both studies the values of cytokines are lower as compared to those of our study.

The rising incidence of T2DM and obesity in Pakistani population is alarming. Pakistan will be fourth country with largest number of diabetics by the year 2030[2]. There is paucity of data about role of inflammation in T2DM. Our study is the first one regarding role of cytokines in Pakistani diabetic population. In-depth investigations at genetic level are required as there are inter-ethnic variations in the levels of cytokines and their association with BMI and insulin resistance. Our study supports the evidence that higher levels of inflammatory cytokines are related to pathogenesis of T2DM and play role in insulin resistance.

CONCLUSION

There is augmented inflammation in T2DM in Pakistani patients which plays role in higher insulin resistance in these patients. TNF- α levels increases with longer duration of the disease. Levels of inflammatory markers are not correlated to BMI, dyslipidemia or increasing age. This is the first study reporting correlation of inflammatory cytokines with type 2 diabetic Pakistani patients.

REFERENCES

1. Ginter E, Simko V. Diabetes type 2 pandemic in 21st century. Bratiisl Lek Listy 2010;111:134-7.

2. Shera AS, Basit A, Fawwad A, Hakeem R, Ahmedani MY, Hydrie MZ, et al. Pakistan National Diabetes Survey: prevalence of glucose intolerance and associated factors in the Punjab Province of Pakistan. Prim Care Diabetes

2010;4:79-83.

3. Kolb H, Mandrup-Poulsen T. The global diabetes epidemic as a consequence of lifestyle-induced low-grade inflammation. Diabetologia 2010;53:10-20.

4. Kolb H, Mandrup-Poulsen T. An immune origin of type 2 diabetes? Diabetologia 2005;48:1038-50.

5. Hoene M, Weigert C. Role of interleukin-6 in insulin resistance, body fat distribution and energy balance. Obes Rev 2008;9:20-9.

6. Glunds S, Krook A. Role of interleukin-6 signaling in glucose and lipid metabolism. Acta Physiol (Oxf) 2008;192:37-48.

7. Kristiansen OP, Mandrup-Poulsen T. Interleukin 6 and Diabetes : the Good, the Bad or the indifferent? Diabetes 2005;54:114-24.

8. Testa R, Olivieri F, Bonfigli AR, Sirolla C, Boemi M, Marchegiani F, et al. Interleukin 6-174G>C polymorphism affects the association between IL-6 plasma levels and insulin resistance in type 2 diabetic patients. Diabetes Res Clin Pract 2006;71:299-305.

9. Hu FB, Meigs JB, Li TY, Rifai N, Manson JE. Inflammatory markers and risk of developing type 2 diabetes in women. Diabetes 2004;53:693-700.

10. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and B-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412-9.

11. Gokulakrishan K, Mohanavalli KT, Monickaraj F, Mohan V, Balasubramanyam M. Subclinical inflammation/oxidation as revealed by altered gene expression profiles in subjects with impaired glucose tolerance and type 2 diabetes patients. Mol Cell Biochem 2009;324:173-81.

12. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Speigelman BM. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. J Clin Invest 1995;95:2409-15.

13. Qi L, van Dam RM, Meigs JB, Manson JE, Hunter D, Hu FB. Genetic variations in IL-6 gene and type 2 diabetes: tagging-SNP haplotype analysis in large-scale case-control study and meta-analysis. Hum Mol Gene 2006;15:1914-20.

14. Nilsson J, Jovinge S, Niemann A, Reneland R, Lithel H. Relation between plasma tumor necrosis factor alpha and insulin sensitivity in elderly men with non-insulin-dependent diabetes mellitus. Arterioscler Thromb Vasc Biol 1998;18:1192-202.

15. Goyal R, Faizy AF, Siddiqui SS, Singhai M. Evaluation of TNF-α and IL-6 in obese and non-obese Diabetics: Pre- and postinsulin effects. N Am J Med Sci 2012;4:180-4.

16. Ruge T, Lockton JA, Renstrom F, Lystig T, Sukonina V, Svensson MK, et al. Acute hyperinsulinism raises plasma interleukin-6 in both non diabetic and type 2 diabetes mellitus subjects, and this effect is inversely associated with body mass index. Metabolism 2009;58:860-6.

17. Stephen JW, Hurel SJ, Lowe GD, Rumley A, Humphries SE. Association between plasma IL-6, the IL-6 -174G>C gene variant and the metabolic syndrome in type 2 diabetes mellitus. Mol Genet Metab 2009;90:422-8.

18. Qi Lu, Van-Dam RM, Meigs JB, Manson JE, Hunter D, Hu FB. Genetic variations in IL-6 gene and type 2 diabetes: tagging-SNP haplotype analysis in large-scale case-control study and meta-analysis. Hum Mol Genet 2006;15:1914-20.

19. Vozarova B, Fernandez-Real JM, Knowler WC, Gallart L, Hanson RL, Gruber JD, et al. The interleukin-6 (-174) G/C promoter polymorphism ia associated with type 2 diabetes mellitus in Native Americans and Caucasians. Hum Genet 2003;112:409-13.

20. Wang Y, Ng MC, So WY, Ma R, Ko GT, Tong PC, et al. Association between tumour necrosis factor-alpha G308A polymorphism and risk of nephropathy in obese Chinese type 2 diabetic patients. Nephrol Dial Transplant 2005;20;2733-8.

21. Abassi MA, Mahar SA, Qureshi AA, Sangi S. Association of leukocyte count with albumin excretion in type 2 diabetes mellitus. Med Channel 2006;12:28-31.

22. Dastagir I, Khurshid R. Immune dysfunction in poorly controlled diabetic patients. J Cardiovascular Dis 2003;1:35-7.

23. Khan DA, Ansari WM, Khan FA. Pro/Anti in-

flammatory cytokines in pathogenesis of pre mature coronary artery disease. J Interferon Cytokine Res 2011;31:561-7.

24. Awan ZA, Naveed AK, Malik MM, Naveed A. Evaluation of C-reactive protein and interleukin-6 as markers of coronary artery disease. Ann King Edward Med Uni 2008;14:50-5.

CONTRIBUTORS

AN conceived the idea, planned and wrote the manuscript of the study. AKN designed the study and approved the final draft of manuscript. MMH & SIR assisted analysis and interpretation of data and gave input in the manuscript. All the authors contributed significantly to the research that resulted in the submitted manuscript.