HYPERFIBRINOGENEMIA IN RELATION TO GLYCEMIC STATUS AND DYSLIPIDEMIA IN TYPE 2 DIABETES MELLITUS AND CORONARY HEART DISEASE

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ABSTRACT

OBJECTIVES: To determine plasma fibrinogen level in type 2 diabetic patients with and without coronary heart disease (CHD) and to find relationship of fibrinogen with glycemic control and lipid profile in these patients.

METHODS: A cross-sectional / analytical study was performed among the outdoor and indoor patients visiting Khyber Teaching Hospital and Hayatabad Medical Complex, Peshawar, Pakistan. The study consisted of two groups. Group A was comprised of 100 type 2 diabetic patients without CHD and group B was comprised of 100 type 2 diabetic patients with CHD. Biochemical analysis of samples was carried out in the Research laboratory of Biochemistry Department, Khyber Medical College, Peshawar. Plasma fibrinogen level was measured by clotting method and statistical analysis was done on SPSS 19 soft ware.

RESULTS: Mean plasma fibrinogen level was 413.44±139.75 mg/dL & 478.63±165.97 mg/dL was observed in group-A & group-B respectively (p<0.05). Mean fasting blood sugar was 175.61±59.13 mg/dL & 213.80±91.62 mg/dL in group-A & group-B respectively (p<0.05). Mean HbA1C in group-A and group-B was 8.308±3.03% & 10.166±3.452% respectively (p<0.05). A strong positive association of fibrinogen was seen with serum triglycerides (p<0.01), total cholesterol (p<0.05) and body mass index (p<0.01).

CONCLUSION: Fibrinogen levels are elevated in type 2 diabetic patients with and without coronary heart disease. Moreover, hyperfibrinogenemia is associated with serum cholesterol, serum triglycerides and BMI in these patients.

KEY WORDS: Fibrinogen (MeSH); Diabetes Mellitus (MeSH); Hemoglobin A, Glycosylated (MeSH); Body Mass Index (MeSH); Hyperlipidemias (MeSH); Cholesterol (MeSH); Triglycerides (MeSH)

INTRODUCTION

Diabetes mellitus is a group of metabolic disorders that share the common symptom of hyperglycemia due to disturbances in carbohydrate, fat and protein metabolism.[1] Inadequate insulin secretion and insulin resistance have a crucial role in development of type 2 diabetes mellitus (T2DM)[2]. The prevalence of T2DM has increased dramatically over the past two decades and is considered to rise in future all over the world.[3,4] The increased incidence of cardiomyopathies, coronary heart disease and congestive cardiac failure have reduced the overall life expectancy by about 5-10 years in type 2 diabetic patients as compared to non diabetic subjects.[5] The chance of sudden death is 50% more in diabetic subjects than non diabetic subjects and the course is more silent due to autonomic neuropathies.[6,7] Current evidence suggests that inflammation, endothelial dysfunctions and increased thrombotic tendency are strongly associated with coronary heart disease in type 2 diabetes mellitus.[8-10]

The increased incidence of cardiovascular morbidity and mortality in type 2 diabetic patients could not be clearly explained by classical risk factors such as obesity, cigarette smoking, hypercholesterolemia and hypertension. So that's why special attention is being paid to identify the defects in haemostatic mechanisms.[11]

Fibrinogen is a 340 KDa large glycoprotein molecule secreted by hepatocytes and acts as a natural substrate for thrombin and an acute phase reactant.[12-14] Fibrinogen plays a key role in the process of inflammation, maintenance of hemostasis, tissue repair and angiogenesis. It mediated coagulation cascade and inflammation by: (i) converting into fibrin by thrombin (ii) modulating adhesion of neutrophils, platelets and endothelial cells to fibrinogen at the site of injury.[15-17] Type 2 diabetic patients with insulin resistance show high levels of inflammatory markers like fibrinogen.[18,19]

As already mentioned diabetic patients have 2 to 4 times higher risk for coronary heart disease and traditional cardiovascular risk factors also associated with diabetes mellitus do not fully explain this extra risk.[20] Fibrinogen is not only a major circulating coagulation protein

but is also the precursor of fibrin. It has a key role in determination of blood viscosity and platelet aggregation. The plasma fibrinogen level may be lowered to a considerable extent through life style interventions thus affecting the levels of established risk factors such as exercise, weight reduction, cessation of smoking and alcohol and leading to a possibility that regular measurement and modification of fibrinogen may prove helpful in disease prediction or prevention in high risk subjects.[21]

In Pakistan fewer studies are available on the subject of inflammatory markers in diabetes mellitus and to our knowledge fibrinogen level in diabetic subjects of KPK has not been previously studied.

Aim of the present study was to determine the level of plasma fibrinogen in type 2 diabetic subjects with and without coronary heart disease in the region of KPK. Furthermore; it was also intended to find relationship of fibrinogen level with dyslipidemia and glycemic status of these subjects.

METHODS

A cross-sectional / analytical study was conducted among the outdoor and admitted patients of Hayatabad Medical Complex and Khyber Teaching Hospital, Peshawar. Biochemical analysis of samples was carried out in the Research Laboratory of Biochemistry Department, Khyber Medical College, Peshawar. Study population was comprised of two groups. Group A contained 100 type 2 diabetic patients and Group B contained 100 type 2 diabetic patients with coronary heart disease.

Inclusion criteria: All those type 2 diabetic patients who had the disorder for at least four years were included in group A while all those type 2 diabetic patients who had first attack of myocardial infarction were included in group B.

Exclusion criteria: Patients with liver dysfunction, thyroid disorders and those using oral contraceptive pills or lipid lowering drugs were excluded from the study.

The approval of the study was obtained by Institutional Ethical Research Board (IERB) of Khyber Medical College, Peshawar. After an overnight fasting under aseptic techniques, fasting blood sugar (FBS), total cholesterol (TC), triglycerides (TG), high density lipoproteins cholesterol (HDL-C), glycosylated hemoglobin (HbAlc) and plasma fibrinogen were measured.

Biochemical analysis

All participants provided 5 mL of fasting venous blood samples under aseptic conditions. Blood samples were centrifuged for 5 minutes at 4000 rpm and clear serum was obtained.

FBS and lipid profile were determined on semi auto-chemistry analyzer Metrolab 1600 DR by enzymatic colorimetric method on Kits of Eli Tech diagnostic, France. Ion-exchange resin colorimetric method was used to determine glycosylated hemoglobin using kit of Human Diagnostic Germany. Plasma fibrinogen was measured by clotting method using kit of FIBRI- PRESET Diagnostic Stage S.A.S of France. In functional clotting method of fibrinogen estimation thrombin is added to citrated plasma leading to its coagulation. The coagulation time is directly proportional to concentration of fibrinogen in the sample. This type of assay is recommended for measurement of fibrinogen level when dysfibrinogenemia is suspected.[22]

Statistical analysis:

Data was analyzed with SPSS version 19. Results were expressed as mean ± SD (standard deviation). Comparison of variables between the groups was done using student's t test. Results having p value <0.05 were considered significant. Association of fibrinogen with different variables was found using Pearson correlation coefficient r.

RESULTS

Table I indicates the biochemical profile of studied population. Group B patients showed a significant increase (P<0.05) in age as compared to Group A patients. However no statistical difference was found on looking for body mass index and hypertension between the patients of two groups. FBS, HbAlc and fibrinogen levels were significantly higher (P<0.05) in patients of Group B as compared to Group A.

Table II shows correlation of fibrinogen with various variables separately in both groups. A significant positive (P<0.05) correlation of fibrinogen with BMI was present in Group A patients. While a highly significant positive (P<0.01) correlation was found between fibrinogen and triglycerides in group B patients.

TABLE I: DEMOGRAPHIC, CLINICAL AND BIOCHEMICAL CHARACTERISTICS OF THE TWO GROUPS

Parameters	Group-A	Group-B	P-value
	Mean±SD	Mean±SD	
FBS* (mg/dL)	175.61±59.13	213.80±91.62	<0.05
HbA1C** (%)	8.308±3.03	10.166±3.452	<0.05
TC*** (mg/dL)	272.06±121.15	279.61±151.82	NS$$
TG# (mg/dL)	290.02±203.23	324.37±142.60	NS$$
HDL-C## (mg/dL)	44.32+15.24	40.67±16.53	NS$$
LDL-C$(mg/dL)	171.58±125.13	176.09±154.09	NS$$
Fibrinogen (mg/dL)	413.44±139.75	478.63±165.97	<0.05

*Fasting Blood Glucose , **Glycosylated Haemoglobin, ***Total Cholesterol , #Triglycerides, ##High Density Lipoprotein-Cholesterol, $Low Density Lipoprotein-Cholesterol., $$Non-Significant

TABLE II: CORRELATION OF FIBRINOGEN WITH DIFFERENT PARAMETERS IN GROUP A AND GROUP

Parameters	Group-A	Group-B
	r	r
Age (years)	-.080	-.102
Systolic blood pressure (mmHg)	.047	.008
Diastolic blood pressure (mmHg)	.057	.165
Body mass Index (Kg/m^2)	.241*	.134
Fasting Blood Glucose (mg/dL)	.116	-.063
HbA1C (%)	.041	.058
Total Cholesterol (mg/dL)	.165	.119
Triglycerides (mg/dL)	.078	.443**
High Density Lipoprotein-Cholesterol (mg/dL)	-.012	-.071
Low Density Lipoprotein-Cholesterol (mg/dL)	.144	.047

*(P<0.05), * *(P<0.01)

TABLE III: CORRELATION OF FIBRINOGEN WITH DIFFERENT PARAMETERS IN THE TWO GROUPS AS A WHOLE

Parameters	Group-A	Group-B
	r	r
Age (yrs)	-.015	NS
Systolic blood pressure (mmHg)	.008	NS
Diastolic blood pressure (mmHg)	.121	NS
Body mass Index (Kg/m^2)	.187**	<0.01
Fasting Blood Glucose (mg/dL)	.050	NS
HbA1C (%)	.105	NS
Total Cholesterol (mg/dL)	.140*	<0.05
Triglycerides (mg/dL)	.250**	<0.01
High Density Lipoprotein-Cholesterol (mg/dL)	-.067	NS
Low Density Lipoprotein-Cholesterol (mg/dL)	.088	NS

*(P<0.05), * *(P<0.01)

Table III shows correlation of fibrinogen with different parameters collectively in the studied population. On looking for association of fibrinogen with BMI and triglycerides, a strong significant (P<0.01) positive association was obtained. Total cholesterol also showed a significant (P<0.05) positive association with fibrinogen.

DISCUSSION

Myocardial infarction and stroke are the major thrombotic complications of type 2 diabetes mellitus and emerging causes of morbidity and mortality all over the world.[23] Many studies have established the role of atherosclerosis, endothelial injury, smooth muscle cell proliferation and inflammation in the development of coronary heart disease in type 2 diabetic subjects.[24,25] The role of hyper-coagulability and plasma fibrinogen in this complex situation has been identified by various clinical, experimental and genetic studies stating that defects occurring in coagulation and fibrinolytic pathways in diabetic subjects make them more vulnerable for development of CHD.[26,27]

Atheromatous plaques have been shown to contain fibrinogen and its degradation product, fibronectin, which play a role in cell proliferation. Fibrinogen is mainly involved in the mechanism of platelet aggregation, thrombus formation and plasma viscosity.[26,28]

Diabetics are at a greater risk to develop premature atherosclerosis. Moreover, they are more prone to develop thrombi due to hyper-reactivity of platelets, increased activation of prothrombotic coagulation factors and decreased fibrinolysis.[29,30]

In this study, plasma fibrinogen level was significantly high (P< 0.05) in type 2 diabetic patients with coronary heart disease. Hong et al[31] showed a strong association between raised plasma fibrinogen level and increased risk and severity of CHD in type 2 diabetic patients. They based this observation on the relationship between increased fibrinogen level and long term glycolipid abnormalities as well as presence of long standing low grade inflammation leading to atherosclerotic plaques. Same results were also reported by Bosevski et al[32] and EPIC –Norfolk study[33].

Madhu et al[34] also reported the same observations after comparing three groups containing control and diabetic patients with and without CHD. Progressively high level (P <0.05) of fibrinogen was found in control, diabetic patients without CHD and diabetic patients with CHD. Bembdi et al[35] reported high levels of plasma fibrinogen (P< 0.05) in type 2 diabetic patients than normal healthy controls.

Some studies have failed to establish any correlation between fibrinogen and coronary heart disease.[36,37] Lowe et al[36] conducted largest prospective study to date trying to evaluate a relationship with inflammatory markers including fibrinogen and the risk of coronary heart disease in type 2 diabetics. They recruited 4197 type 2 diabetic patients with CHD from 20 different countries belonging to Asia, Australia, Europe and

North America with age ≥55 years. They concluded that fibrinogen although raised had no predictive role of micro and macrovascular events or mortality in the study population.

Our results showed higher level of HbAlc in type 2 diabetic patients with CHD. Multiple metabolic cascades are involved in glucose metabolism in diabetes mellitus e.g. hexosamine pathway, advanced glycation end products, polyol pathway and protein kinase C.[38] Hyperglycemia causes glycosylation of proteins especially LDL-C, making them more susceptible for oxidation, and enhances atherosclerosis. The positive relationship of hyperglycemic status and development of CHD in type 2 diabetes mellitus has been observed by Chennai-urban population study.[39]

In this study we have seen a positive association of fibrinogen with triglycerides (TG) and total cholesterol (TC). Linear trends of fibrinogen with age, total cholesterol, triglycerides and body mass index was reported by Stec et al.[40-42]

A positive association of fibrinogen with body mass index was observed in this study which is in tune with the observations reported by Bembdi et al[35] and Stec et al.[40] However these results are not consistent with Lyer et al[26] which may be due to small number of subjects in the study.

The limitations of this study include a relatively small sample size which may have led to non-significant results, and the inclusion of the participants of specific age group (40 and above). Results may be different for individuals of different age groups having different life styles and activity levels. However, the randomized design of the present study is its strength

CONCLUSION

It can be concluded from the study that fibrinogen levels are markedly increased in type 2 diabetic subjects with and without CHD. Plasma fibrinogen level is positively correlated with total cholesterol, triglycerides and BMI in these patients. Thus hyprfibrinogenimia may act as a biomarker of deranged lipid profile in type 2 diabetes mellitus alone and with coronary heart disease.

REFERENCES

1. MN Chatergee, Rana Shinde. Text Book of Medical Biochemistry (seventh edition). Arjuna YN, Jaypee Brothers Medical Publishers, New Delhi, 2007, P 321

2. Pai KJ, Pischon T, Jing MA, Manson JE, Susan EH, Joshipura K, et al. Inflammatory markers and the risk of coronary heart disease in men and women. N Engl Med 2004; 351: 2599-610.

3. Bombed AS. A study of plasma Fibrinogen level in Type-2 Diabetes Mellitus and its relation to Glycemic control. Indian J Hematol Blood Transfuse 2012; 28(2): 105-108.

4. 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.

5. Skyler J. Diabetic complications: the importance of glucose control. Endocrinol Metab Clin North Am 1996; 25: 243-54.

6. Davis TM, Fortun P, Mulder J, Davis WA, Bruce DG. Silent myocardial infarction and its prognosis in a community based cohort of type 2 diabetic patients; the Fremantle diabetes study. Diabetologia 2004;47(3):395-9.

7. Rubin R, Strayer DDS, Rubin E. Rubin's Pathology: Clinicopathological Foundations of Medicine. Maryland: Lippincott Williams and Wilkins, 2001, p. 549.

8. Sanidas EA, Vauranakis M, Papaioannou TG, Carlier S, Syros G, Dangas G, et al. Study of atheromatous plaque using intravascular ultrasound. Hellenic J Cardiol 2008; 49: 415-21.

9. Vogiatzi G, tousoulis D, Stefanadis C. The role of oxidative stress in atherosclerosis, Hellenic J Cardiol 2009; 50: 402-9.

10. Kampoli A-M, Tousoulis D, Antoniades C, Siasos G, Stefanadis C. Biomarkers of premature atherosclerosis. Trends Mol Med 2009;15:323-32.

11. Papageorgiou N, Tousoulis D, Siasos G, Stefandis C. Is fibrinogen a marker of inflammation in coronary artery diseases? Hellenic J Cardiol 2010; 51: 1-9.

12. De Maat MP. Effects of diet, drugs and genes on plasma fibrinogen level. Ann N Y Acad Sci 2001; 936: 509-21.

13. Ilyas S, Waheed U, Saboor M. Coagulation abnormalities in diabetes mellitus. J Public Health Biol Sci 2013; 2(4):308-12.

14. Lee AJ, Lowe GD, Smith WC, Tunstall-Pedoe H. Plasma fibrinogen in women: relationship with oral contraception, the menopause and hormone replacement therapy. Br J Haematol 1993; 83(4): 616-21.

15. 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.

16. Laurens N, Koolwijhk P, de Maat MP. Fibrin structure and wound healing. J Thromb Haemost 2006; 4: 932-9.

17. Naito M, Funaki C, Hayashi T, Yamada K, Asai K, Yoshimine N et al. Substrate bound fibrinogen, fibrin and other cell attachment-promoting proteins as a scaffold for cultured vascular smooth musckle cell. Atherosclerosis 1992; 96: 227-34.

18. Schulze MB, Rimm EB, Li T, Rifai N, Stamfer MJ, Hu FB. C-reactive protein and incident cardiovascular events among men with diabetes. Diabete Care 2004; 27(4): 889-94.

19. Dandona P, Alijada A. A rational approach to pathogenesis and treatment of type 2 diabetes mellitus, insulin resistance inflammation and atherosclerosis. Am J Cardiol 2002; 90: 27-33.

20. Kafle DR, Shrestha P. Study of fibrinogen in patients with diabetes mellitus. Nepal Med Coll J 2010; 12(1):34-7.

21. Cristobal JJR, Grote CA, Mercade PT, Santos JMP, Sendra EP, Lloret AM et al. Randomised clinical trial of an intensive intervention in the primary care setting of patients with high plasma fibrinogen in the primary prevention of cardiovascular disease. BMC Research Notes 2012; 5: 126-32.

22. Guidelines on fibrinogen assays. Br J Haematol 2003: 121 (3): 396-404.

23. Sjohoim A, Nyström T. Endothelial inflammation in insulin resistance. Lancet 2005; 365: 610-2.

24. Venkataramana G, Indira P; Rao DVM. Changes of plasma total proteins, albumin and fibrinogen in type 2 diabetes mellitus: A pilot study. Indian Journal of Basic & Applied Medical Research; June 2013: Issue-7, Vol.-2, P. 679-85.

25. Fuster V, Badimon L, Badimon J. Chesebro J. The pathogenesis of coronary artery disease and the acute coronary syndromes. N Engl J Med. 1992; 326: 242-50.

26. Lyer MU, Desai P. Assesment of C-reactive protein and fibrinogen level in type 2 diabetes mellitus. Biomed Res 2010;

21: 208-13.

27. Alzahrani SH, Ajjan RA. Coagulation and fibrinolysis in diabetes. Diab Vasc Dis Res 2010; (4): 260-73.

28. Mohamed RA, Abdrabo AA, Muddathir AM. The Association between Microalbuminuria and Plasma Fibrinogen Levels in type 2 diabetic Sudanese patients. American Journal of Research Communication, 2013, 1(10): 69-75.

29. Stratmann B, Tschoepe D. Atherogenesis and atherothrombosis-focus on diabetes mellitus. Best Pract Res Clin Endocrinol Metab 2009; 23: 291-303.

30. Martin-Timon I, Collantes SC, Galindo AS, Gomez FJC. Type 2 diabetes and cardiovascular disease: Have all risk factors the same strength? World J Diabetes 2014; 5(4): 444-70.

31. Hong LF, Li XL, Luo SH, Guo YL, Zhu CG, Qing P, et al. Association of fibrinogen with severity of stable coronary artery disease in patients with type 2 diabetes mellitus. Disease Markers 2014, Article ID 485687, 8 pages. http:// dx. Doi. Org/10.1155/2014/485687.

32. Bosevski M, Bosevska G, Stojanovska L. Influence of fibrinogen and C-RP on progression of peripheral arterial disease in type 2 diabetes: a preliminary report. Cardiovascular Diabetology 2013; 12: 29-34.

33. Rana JS, Cote M, Despris JP, Sandhu MS, Talmud PJ, Ninio E, et al. Inflammatory biomarker and the prediction of coronary events among people at intermediate risk the EPIC–Norfolk prospective population study. Heart 2009; 95: 1682-7.

34. Madhu SV, Kumar V, Gambhir JK. Plasma fibrinogen in patients with type 2 diabetes mellitus with and without macrovascular disease and its relationship with endothelial function, carotid intima media thickness and dyslipidemia. Int J Diabetes In Developing Countries. 2011; 31(3): 143-8.

35. Bembdi AS. A study of plasma fibrinogen level in type-2 diabetes mellitus and its relation to glycemic control. Indian J Hematol Blood Transfus 2012; 28(2): 105-8.

36. Lowe G, Woodward M, Hillis G, Rumley A, Li Q, Harrap S, et al. Circulating inflammatory markers and the risk of vascular complications and mortality in people with type 2 diabetes and cardiovascular disease or risk factors: The ADVANCE Study. Diabetes 2014;63(3): 1115-23.

37. Lawlor DA, Smith GD, Rumley A, Lowe GDO, Ebrahim S. Association of fibrinogen and C-reactive protein with prevalent and incident coronary heart disease are attenuated by adjustment for confounding factors. British Women's Heart and Health study. Thromb Haemost 2005; 93: 955-63.

38. Theodore Mazzone. Intensive glucose lowering and cardiovascular disease prevention in diabetes: Reconciling the recent clinical trial data. Circulation 2010; 122: 2201-11.

39. Mohan V, Venkatraman JV, Pradeepa R. Epidemiology of cardiovascular disease in type 2 diabetes: the Indian scenario. J Diabetes Sci Technol 2010; 4(1): 158-70.

40. Stec JJ, Silbershatz H, Tofler GH, Matheney TH, Sutherland P, Lipinska L, et al. Association of fibrinogen with cardiovascular risk factors and cardiovascular disease in the Framingham off spring population. Circulation 2000; 102: 1634-8.

41. Ernst E, Resch KL. Fibrinogen as a cardiovascular risk factor. A meta analysis and review of the literature. Ann Intern Med 1993; 118: 956-63.

42. Kaptoge S, Angelantonio ED, Pennells L, Wood AM, Ian R, Peri G, et al. C-reactive protein, fibrinogen and cardiovascular disease prediction. New Engl J Med 2012; 367: 1310-20.

AUTHOR'S CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under:

SA: Concept & study design, acquisition analysis and interpretation of data, drafting the manuscript, final approval of the version to be published

MuAK: Critical revision, drafting the manuscript, final approval of the version to be published,

MoAK: Acquisition analysis and interpretation of data, drafting the manuscript, final approval of the version to be published

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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