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RESEARCH ARTICLE

ASSOCIATION OF D-DIMER IN TYPE 2 DIABETES MELLITUS.

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Key words:-
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Abstract

Context: Diabetes mellitus is a leading cause of vascular morbidity. Etiology of diabetic vascular complications is multifactorial. Alteration of blood coagulation and fibrinolysis along with poor glycaemic control in diabetes has also been implicated in the development of diabetic vascular complications. Plasma level of D-Dimer reflects the amount of lysed cross-linked fibrin and hence is an accepted marker of hypercoagulability.

Aim: The aim of this study is to evaluate the clinical significance of plasma D-dimer in type 2 Diabetes Mellitus.

Settings and Design: This study is a cross sectional study.

Methods and Material: A total of 90 patients were selected based on clinical status and divided into 3 groups: 30 patients of newly detected type 2 DM, 30 patients having diabetic nephropathy and 30 patients of DM with coronary artery disease. Plasma Ddimer was measured using Second-generation latex agglutination (immunoturbidimetric) method in Cobas Integra 400 plus. The lipid profile and HbA1c were also measured.

Statistical analysis used: Statistical analysis was performed by one way ANOVA, scheffe post hoc test and independent t- test.

Results: Patients with diabetic nephropathy had significantly higher plasma D-dimer levels (2.48±1.28µg/mL) than patients without complications (0.28±0.12 µg/mL) ($p < 0.0001$). There were significantly higher plasma D-dimer levels in patients with coronaryartery disease (2.05±1.06 µg/mL) than patients without complications (0.28±0.12 µg/mL) ($p < 0.0001$). And there is no significant different in lipid profile in DM with or withoutcoronary artery diseases.

Conclusions: Our data suggest that in DM patients presenting with coronary artery diseases without dyslipidemias, the D-Dimer can serve as a novel marker for prediction of the risk of coronary artery diseases and higher level of D-Dimer in diabetic nephropathy patients suggest that increased thrombogenic state may be related to increased susceptibility of vascular disease in these patients.

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Introduction:-

Diabetes mellitus comprises a group of common metabolic disorders that sharethe phenotype of hyperglycemia. In patients with diabetes mellitus having vascularcomplications, control of blood glucose levels is a crucial factor in determiningprognosis. The mechanisms that mediate vascular complications in diabetic patients arenot at fully understood. Etiology of diabetic vascular complications is multifactorial.

Alteration of blood coagulation and fibrinolysis along with poor glycemic control indiabetes has also been implicated in the development of diabetic complications. Type 2diabetes mellitus is a growing cause of disability and premature death, mainly because ofcardiovascular diseases and chronic complications[1].

Prolonged exposure to hyperglycemia is now documented in the pathogenesis ofdiabetic complications like nephropathy and cardiovascular disease (CVD):

1. Glycated proteins and lipids which can interfere with their normal function bydisrupting the molecular conformation, alter enzymatic activity, reduce degradative capacity and interfere with receptor recognition. In addition, the glycated proteins interactwith specific receptors present on all cells relevant to the atherosclerotic process which includes monocyte-derived macrophages, endothelial cells, and smooth muscle cells. Theinteraction of glycated proteins with their receptor results in the induction of oxidativestress and proinflammatory responses[2].
2. Oxidative stress could further aggravate complications[2].
3. Protein kinase C (PKC) activation by prolonged hyperglycemia and glycated proteinwith subsequent alteration in growth factor expression. Importantly, these mechanisms may be interrelated. For example, hyperglycemia induced oxidative stress promotes boththe formation of advanced glycosylation end products (AGE) and PKC activation.

This study was undertaken to investigate the role of coagulation/fibrinolysis in thepathogenesis of diabetic complications by evaluating the association of d-dimer in type 2DM.

Materials & Method:-

The study was carried out at Shree Krishna Hospital and H M Patel Centre for Medical Care and Education, a 550 bedded tertiary care rural based, teaching hospital attached to Pramukh Swami Medical College, Karamsad, from September 2013 to October 2014. A study protocol was set before undertaking this study and it was approved by the Institutional Human Research Ethical Committee.

This was a cross sectional study consisted of 90 individual above the age of 40 years areincluded divided into three group. All the participants in the study were explained clearly about the purpose and the nature of the study in the language best understood by them. The samples were drawn from the participants only after obtaining a written and informed consent.

Out of this the group-1 consisted of 30 patients of newly detected type 2 DM who wererecruited from those attending medical outpatient department and health check-updepartment. Group-2 consisted of 30 patients having diabetic nephropathy, whose serumcreatinine was >1.4mg/dl, admitted in medical intensive care unit were recruited forstudy and group 3 consisted of 30 patients of type 2 DM admitted in emergency andcardiac ICU within 6 hrs of clinical signs and symptoms of acute coronary syndromeswithout any history of diabetic nephropathy were included in this study. The patients hadbeen diagnosed depending upon the assessment as per the clinical symptoms such aschest pain with or without radiation, chest heaviness, shortness of breath, lower jaw pain,left arm pain, epigastric pain, palpitations and signs like hypotension and ECG changessuggestive of MI. The samples were collected from group 3 patients before anythrombolytic treatment. Patients were considered to be diabetic based on ADA criteriafor the diagnosis of diabetes mellitus[16].

All the patients with Liver disease, Malignancy, Trauma, Pregnancy, Pulmonaryembolism, Disseminated Intravascular Coagulation were excluded from study to avoidfalse positive result of D-Dimer. The study protocol was set before undertaking this studyand it was approved by institutional Human Research Ethics Committee. Plasma D-dimerwas measured using Second-generation latex agglutination (immunoturbidimetric)method[17], HBA1c was measured using Immunoturbidimetry Standardized according toIFCC method[18], creatinine was measured using Colorimetric Compensated Jaffe kineticmethod [19], Triacylglycerol was measured using Colorimetric Endpoint GPO-POD Method[20],Cholesterol was measured using Colorimetric Assay with Endpoint CHOD-POD Method[21]and HDL-

Cholesterol was measured using Homogenous Enzymatic Colorimetric Assay[22]in Cobas Integra 400 plus. Analysis was performed using the commercially availablestatistical software SPSS – 14.0 version and Microsoft excel. Values are given as mean±SD. Comparison of Renal function tests, HbA1c, D-dimer in three groups of patients byONE WAY ANOVA and pairwise mean comparisons by Post hoc (scheffe test).

Comparison of lipid profile in DM patients with or without coronary artery diseases byindependent t-test. The p value of less than 0.05 was considered statistically significant.

Result:-

The Patients enrolled for the study were in the age group of 40 to 80 years. The meanage of the diabetic patient without complication, diabetic with nephropathy and diabeteswith coronary artery diseases was 55.43±9.57, 60.93±10.57 & 62.1±78yearsrespectively.

The mean ± SD of duration of diabetes in the group 1 individuals is less than one year,whereas it is 8.64±2.34 years in group 2 and 9.28±2.88 years in the group 3.

ONE WAY ANOVA:-

Table1:- Comparison of Renal function tests, HbA1c, D-dimer in three groups ofpatients

Parameter	DWC (Group-1)	DN (Group-2)	DM+CAD (Group-3)	ANOVA P VALUE
HbA1c Mean±SD	8.24±1.74	8.68±2.3	9.17±2.34	0.247
Creatinine(mg/dl) Mean±SD	0.73±0.23	3.45±2.29	0.95±0.27	<0.0001
D-Dimer(µg FEU/mL) Mean±SD	0.28±0.12	2.48±1.28	2.05±1.06	<0.0001

Using ANOVA p-value we conclude that

1. There is no significant difference in atleast one pair of mean of HbA1c among all threegroups.
2. There is difference in atleast one pair of mean of creatinine among the three groups.
3. There is difference in atleast one pair of mean of D-Dimer among the three groups.
4. Post hoc (scheffe test) revealed that there is no statistically significant difference betweenmean HbA1c in either a pair

Table 2:- Pairwise mean comparisons of D-DIMER among three groups

Parameter	Mean±SD of D-Dimer (µg FEU/mL)	P-value
DWC (Group-1)	0.28±0.12	<0.0001
DN (Group-2)	2.48±1.28	
DWC (Group-1)	0.28±0.12	<0.0001
DM+CAD (Group-3)	2.05±1.06	
DN (Group-2)	2.48±1.28	0.230
DM+CAD (Group-3)	2.05±1.06	

Post hoc (scheffe test) revealed that:-

1. Mean D-Dimer (µg FEU/mL) is statistically significantly higher in the diabetic withnephropathy patient as compared with diabetic patient without complication(P=<0.0001).
2. Mean D-Dimer (µg FEU/mL) is statistically significantly higher in the diabetic with
3. coronary artery patient as compared with diabetic patient withoutcomplication(P=<0.0001).
4. There is no statistically significant difference between Mean D-Dimer (µgFEU/mL) in the diabetic with coronary artery patient and Mean D-Dimer (µgFEU/mL) in the diabetic patient with diabetic nephropathy(p=0.230).

T Test:-

Table 3:- Comparison of lipid profile in Diabetic with and without CAD.

Parameters	DWC (Group-1)	DM+CAD (Group 3)	P value
Total cholesterol(mg/dl) Mean±SD	171.43±33.65	168.46±53.00	0.797
Triglyceride(mg/dl) Mean±SD	153.03±73.95	156.66±69.53	0.845
HDL(mg/dl) Mean±SD	42.43±11.39	35.86±8.16	0.013
LDL(mg/dl) Mean±SD	98.26±30.67	101.26±43.44	0.758

Independent sample t test revealed that

1. Mean HDL (mg/dl) is statistically significantly lower in diabetic with coronary artery
2. disease subjects than the diabetic without complication individuals.
3. Mean total cholesterol (mg/dl) (p=0.797), mean triglyceride (mg/dl) (p=0.845) andMean LDL (mg/dl) (p=0.758) are not statistically significant between diabetic patientwithout complication and diabetic with coronary artery disease individuals.

Discussion:-

Comparison of HbA1c:-

In the present study, the mean HbA1c value in diabetes without complications was 8.24±1.74. The mean HbA1c value in diabetic with nephropathy was 8.68±2.3 and in DM with coronary artery disease was 9.17 ±2.34. This was statistically not significant with P value 0.247.

Takashi Yamada et. al.[7] observed that there was no statistically significant difference in HbA1c values in patients with (6.6±1.0) or without (7.3±1.6) microvascular complications, P value being 0.72. The comparison of the patients with (7.0±1.5) and without (7.1±1.4) macrovascular complications also did not show any significance difference, P value being 0.202.

The HbA1c is effective in monitoring long term glucose control in patients with diabetes mellitus. The complications of diabetes not only depend on the duration of diabetes mellitus but also on the long lasting poor glycemic control as indicated by a high level of HbA1c. Hence, there is requirement of a marker for monitoring long term diabetic complications.

On the basis of the knowledge of coagulation/fibrinolysis imbalance in DM, the observation of plasma D-dimer in complicated DM patients, we can use at least complimentary role of D dimer in early identification of complications.

Comparison Of D-Dimer:-

Diabetes mellitus without complication:-

In my study diabetes patients without complications with mean age of 55.43±9.57 years, the d dimer level was 0.28±0.12 µg FEU/mL which was similar to the study by Yolanda Lopez et al[8] of diabetic patients, under metabolic control and presented with no signs of retinopathy, neuropathy or nephropathy (corresponding to our group 1 with a mean age of 54.3±15.2 years), the concentration of D-Dimer was within the normal range 267.10±25.8 µg/L. This value when converted to the units used in our study is 0.26 µgFEU/mL.

Diabetic with coronary artery diseases

When we compared plasma D-dimer levels between diabetes without complications (group 1) and diabetic with coronary artery diseases (group 3), plasma D-dimer levels were found to be significantly ($p<0.0001$) higher in diabetic patients with coronary artery diseases. Our results are similar to other studies[9,10].

Tariq A Zafar(2010)[10] found that significant high plasma D-dimer levels in diabetic patients with myocardial infarction, compared with diabetic patients without myocardial infarction and the control group (445 ± 352 µg/L, 340 ± 249 µg/L, and 156 ± 107 µg/L respectively). D-dimer levels of patients with myocardial infarction were significantly higher than in the patients without myocardial infarction and the control group (P = 0.002 and P = 0.001. respectively).

Ezekiel U. Nwose et al. (2007)[9] in a study 343 participants were selected based on the clinical status and divided into 7 groups: control, family history of diabetes, pre-diabetes with/without CVD, diabetes with/without CVD and CVD without DM. An overall significant difference between groups ($p<0.002$) and a steady rise in D-dimer levels that became increasingly higher than control as the disease progressed from pre-diabetes to cardiovascular complications was observed.

T. Yamada et al [11] in their study observed that the d-dimer level in the diabetic patients without macroangiopathy was 0.63±0.37 µg/ml and with macroangiopathy was 1.12±1.24 µg /ml, this was not statistically significance ($p=0.69$) which is not agreement with my study. In the present study, Diabetic patient with coronary artery disease had total cholesterol level 168.46±53.00, TG level 156.66±69.53, HDL level 35.86±8.16 and LDL level 101.26±43.44. In Diabetic patients without complications the TC level 171.43±33.65, TG level 153.03±73.95, HDL level 42.43±11.39 and LDL level 98.26±30.67 showing only small difference in the lipid profile in the two groups. There was significance difference only in the HDL level ($p = 0.013$).

Our data is similar to Tariq A Zafar et al[10] in which diabetics with MI, TC level being 225±38, TG level 176±81 and HDL level 37±5 and in diabetics without complications, TC level 202±40, TG level 127 ±97 and HDL level 42±7. There are small differences in the lipid profile between the two groups.
It has been reported that over 50% of all future vascular events may occur in persons without dyslipidaemia and that plasma D-Dimer can be a novel risk marker for the prediction of future cardiovascular events in those with normal lipid profile[12].

Our report suggests that besides the involvement of diabetic dyslipidemia in coronary artery diseases, diabetic hypercoagulation is another pathological process in developmentof CAD.

Ezekiel U. Nwose et al. (2007)[9]suggested that laboratory determination of both totalcholesterol & D-dimer could reduce chances of making false negative decision aboutdiabetics with CAD complication.

Diabetic nephropathy:-
When we compared plasma D-Dimer levels between diabetes without complication (group 1) and diabetic with nephropathy (group 2), the plasma D-Dimer levels were found to be significantly ($p<0.0001$) high in latter group. Our results are similar as other studies[4,13,14,15.]

Ichiro Wakabayashi et al.[4] they observed that the mean level of logconverted D-Dimer after adjustment for age and sex was significantly higher in subjects with microalbuminuria ($p<0.01$) than in those with normoalbuminuria. They concluded that the D-Dimer is associated with microalbuminuria in patients with diabetes and this suggests that glomerular dysfunction is in part mediated by hypercoagulability.

J. W. J. van Wersch et al.*(*1991*)* [13] in a study including 116 patients with type 1 DM foundthat the presence of lower HDL-cholesterol, higher triacylglycerols and the elevation of fibrin monomers and D-Dimers is more pronounced in the microalbuminuria group.

Takashi Nagai et al. (1993)[7]studied NIDDM with and without nephropathy along with normal subjects and observed elevated levels of D-Dimer in nephropathy patients.

Kano Y, Kobayashi K et al [14]In their study had been performed in 18 diabetic nephropathy patients and 16 hypertensive patients with nephrosclerosis reported higher level of D-Dimer in both group.

CihangirEremet al[15] studied diabetic with or without nephropathy and observed no significance difference in D-Dimer.

T. Yamada et al [11] in this study d-dimer level in patients without diabetic microangiopathic was 0.54±0.17 mcg/ml and with microangiopathic 0.88±0.90 mcg/ml there was not statistically significance ($p=0.264$) this is not similar to my study.

In Diabetics pathophysiological changes start before development of complications and severity of vascular complications was concordant with microangiopathies (eg.DN) and macroangiopathies (eg.CAD) in the patients

with an increased coagulation/fibrinolysis abnormality. Identification of circulating risk molecules (plasma D-dimer) may help to identify diabetics with high risk of vascular complications. Plasma D-dimer test is now routine and can measure easily. So we can use complimentary test in early identification of complications.

Conclusion:-

In the present study, consisted of 90 patients of type 2 DM in the age of 40-80 years and the complication started after 6 years duration of diabetes. The study showed equal distribution of diabetes and its complication in male and female. If DM patients present with coronary artery diseases, without dyslipidemias, the D-Dimer can serve as a novel marker for prediction of the risk of coronary artery diseases. D-Dimer if employed as an additional test may improve the risk assessment for early coronary artery diseases in DM patients. In our study there is a higher concentration of D-Dimer in coronary artery diseases. We also got a higher level of D-Dimer in diabetic nephropathy patients. It indicates increased procoagulant activity in diabetic nephropathy patients. It is reasonable to assume that the higher level of D-dimer is primarily the result of increased fibrin clot formation and subsequent breakdown. The increased thrombogenic state may be related to increased susceptibility to vascular disease in these patients. D-Dimer, hence, can be used as an additional, novel biomarker of diabetic complications.

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