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RESEARCH ARTICLE

BIOCHEMICAL MARKERS FOR THE PROGRESSION OF DIABETIC NEPHROPATHY.

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Abstract

Introduction:-Homocysteinemia is an independent risk factor for cardiovascular disease, but information on its association with type 2 diabetes and mild nephropathy is limited. Diabetic nephropathy (DN) is one of the most important microvascular complications of diabetes.

Aims and objectives:- The aim of the study was to find out the relationship between plasma total homocysteine concentration, serum folate and vitamin B12 in diabetic nephropathy in patients with type 2 diabetes mellitus.

Materials and Methods:- The study was conducted on total 90 subjects, out of which 30 type 2 diabetics without nephropathy (group I), 30 type 2 diabetics with nephropathy (group II) and 30 controls (age and sex matched) who were free from any chronic disease. Plasma total homocysteine, folate and vitamin B12 levels were estimated along with routine biochemical parameters and compared with healthy controls.

Results:-Plasma total homocysteine levels were significantly higher ($p<0.001$) in subjects with diabetic nephropathy than without nephropathy and controls.

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Introduction:-

The major clinical objective in the management of diabetes mellitus (DM) is to control hyperglycemia and the specific long term objectives are to prevent micro and macrovascular complications. The control of plasma glucose levels in patients with diabetes mellitus is important in preventing long term complications. Poor glycemic control has been identified as one of the risk factors of microalbuminuria (incipient nephropathy) which hastens the progress of renal disease [1, 2].

Homocysteine (Hcy) is the transmethylation product of the essential sulphur-containing amino acid methionine [3, 4]. Experiments have shown that high concentrations of homocysteine may cause vascular damage [5]. Plasma tHcy concentration is strongly related to renal function [6]. A study in rats identified the kidney as a major site for removal and metabolism of Hcy [7]. Two mechanisms appear to be involved. The main source of Hcy is adenosylmethionine dependent methylation of guanidoacetate to form creatine and its anhydride creatinine [8]. Second, renal function plays a central role for clearance of both creatinine and Hcy [9, 10]. Plasma tHcy concentrations in diabetic patients can be affected by both glomerular hyper- and hypofiltration, which can respectively decrease and increase the tHcy concentrations [11, 12]. However, reduced glomerular filtration rate (GFR) accompanies microalbuminuria (MAU) in the late phase of diabetic nephropathy, and reduction of GFR causes elevation of plasma Hcy levels [13, 14]. These data provide a potential link between microalbuminuria and diabetic nephropathy [15-17]. This study was designed to assess the association between plasma tHcy for the evaluation of renal function in type 2 diabetic patients.

Materials and Methods:-

The study was conducted on total 90 subjects, out of which 30 type 2 diabetics without nephropathy (group I), 30 type 2 diabetics with nephropathy (group II) and 30 controls (age and sex matched) who were free from any chronic disease as ascertained by detailed history, physical examination and routine laboratory investigations. An informed consent was obtained from the patients and all the ethical issues were duly taken care of. The diagnosis of diabetes mellitus was based on a previous history of diabetes or the American Diabetes Association (ADA) criteria [18]. Exclusion criteria were: previously recorded myocardial infarction, angina pectoris according to Rose questionnaire [19], stroke, coronary artery by-pass surgery, percutanous transluminal coronary angioplasty, uncontrollable hypertension (blood pressure ≥ 180/ 100 mmHg), medications like digitalis or drugs affecting homocysteine metabolism (including vitamins), peripheral vascular disease, smoking or alcohol consumption, hepatic and/or thyroid disease. The diagnosis of diabetic nephropathy was made by high albumin excretion in urine along with raised levels of fasting plasma glucose (FPG) and glycated hemoglobin (HbA1c). The vascular complications in group II were ruled out by conducting renal function tests including microalbuminuria (< 30 mg/l) and fundoscopic examination which were found to be within the normal range. First voided morning urine samples were analysed for microalbuminuria on Nephelometer (Delta, SEAC) and creatinine by Jaffe's reaction. Fasting heparinised venous blood samples were obtained. HbAıc, creatinine, homocysteine, folate, vitamin B_{12} were determined in blood or serum samples after an overnight fast.
Plasma total homocysteine and serum homocysteine-related vitamins (folate and vitamin B12) levels were measured using chemiluminescence assay.

Serum HbAıc, urea and creatinine were estimated on autoanalyser (Konelab 30i) using standard kit methods (Randox). Statistical analysis was performed using SPSS version 20.0. All the values were expressed as mean± SD and Pearson's coefficient of correlation were used to analyze relationships between biochemical parameters. The level of significance was set as p value < 0.05 as significant.

Results:-

Laboratory findings of patients and control subjects (Mean ± SD) are listed in Table 1. There were no significant differences in age, BMI between the two diabetic groups and the controls. Fasting plasma glucose, HbA1c, plasma homocysteine, vitamin B12 and folate showed significant ($P<0.05$) differences between the two diabetic groups when compared with controls. The levels of urea, creatinine and microalbuminuria were significant in diabetics with nephropathy when compared with diabetics without nephropathy and controls.

Discussion:-

Studies that have examined the relationship between homocysteine levels in patients with diabetes mellitus and the presence of microvascular disease have shown correlation between nephropathy [4, 20]. In early diabetic nephropathy homocysteine levels have been reported to be normal [21]. Hyperhomocysteinemia correlates with both change in glomerular filtration rate (GFR) [22] as well as the presence of microalbuminuria [23- 25].

Plasma tHcy levels in patients with type 2 diabetes with advanced nephropathy were higher than those in control subjects or patients with diabetes without nephropathy. Renal function, represented by creatinine clearance and serum creatinine level, was the strongest contributor to plasma tHcy in patients with diabetes. Microalbuminuria, an early marker of endothelial dysfunction as well as nephropathy, has been reported to be associated with plasma tHcy level in patients with diabetes [15, 26, 27]. However, Smulders et al. [28] recently found no association of microalbuminuria with plasma tHcy levels in patients with type 2 diabetes. We could neither confirm nor reject an association between homocysteine and the development of (micro)albuminuria among NIDDM subjects. These data are in line with previous cross sectional findings [15, 26, 29] and support the hypothesis that homocysteine may play a pathophysiological role in the development of (micro)albuminuria.

However, conflicting results regarding the Hcy level in patients with diabetes have been reported. Some studies found that plasma Hcy levels were increased [4, 30], unchanged [28,31,32], or decreased [33,34] in patients with T2DM.

Some authors found an association between plasma tHcy and microalbuminuria in diabetes [4, 15, 21, 26, 27] while others did not [28,35]. In our study, plasma homocysteine concentrations were significantly higher in

microalbuminuric patients than in normoalbuminuric patients or controls. Chico et al [15], Emoto et al [4], and Lanfredini et al [26] found strong association between homocysteinemia and deteriorated renal function. According to previous reports, homocysteine is ultrafiltrated through the glomeruli, almost completely reabsorbed in the tubuli, and degraded in kidney tissue by transmethylation and transsulfuration [36]. Decreased renal clearance of homocysteine results in homocysteinemia [7-9], but the exact mechanism is unknown [37]. Although kidney function plays an important role in the metabolism of homocysteine, it is not clear whether homocysteinemia is associated with or can precede microalbuminuria [29,38]. Homocysteinemia is believed to increase the risk of atherothrombic disease directly by impairing endothelial function, stimulating vascular smooth muscle cell proliferation, and altering extracellular matrix properties [39]. High plasma homocysteine levels may also exert an atherothrombic effect by inducing oxidative stress, which may impair endothelial function. Oxidative stress is thought to be increased in type 2 diabetes [40]. Thus, homocysteinemia may contribute to the development of renal impairment and subsequent microalbuminuria in diabetic patients through increased oxidative stress [29].

In conclusion, increases in Hcy in diabetic patients are associated with increased complications especially nephropathy. We think that Hcy may not only be a result but also a factor leading to the development of diabetic vascular complications. Since the mechanisms responsible for Hcy-mediated vascular damage still remain obscure, prospective studies are needed to reveal the exact role of hyperhomocysteinemia modified by genetic, dietary and environmental factors in the development of angiopathy in patients with diabetes. Analysis of the results of recent studies that have examined the relationship between homocysteine levels and the presence or absence of macro- and microvas- cular disease in patients with diabetes shows, that for the most part, homocysteine elevation in patients with diabetes mellitus only occurs when renal function deteriorates. The rise in homocysteine does not appear to be linked to the level of glycemic control. In view of the proven relationship between hyperhomocysteinaemia and vascular injury control of plasma homocysteinaemia in patients with diabetes mellitus is best accomplished by management approaches that prevent renal injury rather than by means to reduce homocysteine levels by folate, B12 or betaine supplementation except in those patients who have demonstrated deficiency of these vitamins.

Table 1.Clinical characteristics of type 2 diabetic patients with and without nephropathy (Data represented as Mean ± SD).

Parameters	Healthy controls	Patients with diabetes without nephropathy (GroupI)	Patients with diabetic nephropathy (GroupII)
Number	30	30	30
Age (Years)	52.03±4.45	51.97±4.34	53.50±4.96
BMI (Kg/m^2)	18.38±457.74	28.15±1.25*	30.33±1.33*$^{\#}$
Fasting Plasma Glucose (mmol/l)	4.65±0.30	6.59±0.60*	9.32±0.71*$^{\#}$
HbA1c (%)	4.31±0.53	4.63±0.19*	9.27±0.89*$^{\#}$
Urea (mmol/l)	4.35±1.36	7.14±0.81	10.90±2.52*$^{\#}$
Creatinine (µmol/l)	72.67±10.28	92.08±5.21	113.26±5.08*$^{\#}$
Urine Microalbuminuria (mg/24hr)	14.79±1.73	19.77±3.34	91.90±23.71*$^{\#}$
Homocysteine (µmol/l)	11.63±1.86	17.67±2.04*	33.73±9.16*$^{\#}$
Folate (pmol/L)	11.68±4.15	8.36±1.67*	5.76±2.22*$^{\#}$
Vit B12 (nmol/l)L	217.60±65.45	130.27±17.67*	87.10±9.30*$^{\#}$

***p<0.05 as compared to controls**
$^{\#}$ p<0.05 as compared to group I

Table 2 Pearson's correlation coefficient (r) & p values for determinants of plasma homocysteine in diabetic patients with nephropathy.

Parameters	r	p
Age (yrs.)	0.21	0.262
BMI (Kg/m^2)	-0.230	0.222
Fasting Plasma Glucose (mmol/l)	0.117	0.539
HbA1c (%)	0.408	0.025
Urea (mmol/l)	0.076	0.689
Creatinine (µmol/l)	0.380	0.038
Urine Microalbuminuria (mg/24hr)	0.718	0.000
Folate (pmol/L)	-0.676	0.000
Vit B12 (nmol/l)L	-0.173	0.359

Declaration of Interest:-
The authors declare that they have no conflict of interests

Conclusion:-

The levels of homocysteine were significantly elevated in patients with diabetic nephropathy as compared to diabetic patients without nephropathy. Thus homocysteinemia is associated with type 2 diabetes mellitus and it is irrespective of presence or absence of renal complications and does appear to be linked to glycemic control

List of Abbreviations:-

BMI, Body mass index; HbA1c, Glycosylated Hemoglobin; FPG, Fasting plasma glucose; PPPG, Postprandial plasma glucose; total homocysteine ; tHcy

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