ORIGINAL RESEARCH PAPER

Internal Medicine

A STUDY OF SERUM IL-6 IN PATIENTS OF TYPE-2 DIABETES MELLITUS AND ITS CORRELATION WITH DIABETIC NEPHROPATHY

KEY WORDS: Type-2 diabetes mellitus, Diabetic nephropathy, Interleukin-6, Urinary albumin excretion.

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ABSTRACT

BACKGROUND: Type-2 diabetes mellitus is the most common type of diabetes in the world, accounting for over 90% of cases and posing a substantial global health threat. In type-2 diabetes mellitus, several proinflammatory cytokines generate low-grade inflammation, which is a frequent occurrence in most people. Interleukin-6(IL-6), a proinflammatory cytokine, has been linked to the development of insulin resistance and, as a result, to the pathophysiology of type-2 diabetes mellitus. **METHODS:** All cases of Type-2 Diabetes Mellitus attending the Department of Medicine were screened and a total number of 60 cases that fulfil the inclusion and exclusion criteria were included in the study. A group of 30 healthy controls were taken. **RESULTS:** Out of the 60 subjects of case, i.e. patients with type-2 diabetes mellitus, there were 21(35%) females and 39(65%) males with mean age 49.3+11.9 years . And in the 30 subjects in control group, there were 10(33%) females and 20(67%) males with mean age 52.4+14.2 years. The mean IL-6 of the subjects in case group was 27.2pg/ml and in control group was 2.5 pg/ml with p value <0.0001 . The mean IL-6 values in relation to CKD stages were- 10.22+20.09 pg/ml, 19.5+31.23pg/ml, 32.05+40.39 pg/ml, 47.32+40.13 pg/ml, 55.41+43.25pg/ml, 58.39+46.45pg/ml, in the stages 1, 2, 3A, 3B, 4 and 5 respectively. There was a positive correlation in the values of IL-6 seen with the progression of stages of CKD. **CONCLUSION :** There is a significant difference of IL-6 between diabetic and non diabetic groups, and also a positive relation between IL-6 and urinary albumin excretion as well as severity of diabetic nephropathy.

INTRODUCTION :

Type-2 diabetes mellitus is the most common type of diabetes in the world, accounting for over 90% of cases and posing a substantial global health threat[1,2]. Type-2 diabetes mellitus (T2DM) is on the rise in both emerging and developed countries, posing an epidemic. Diabetes affects more than 62 million Indians, accounting for more than 7.1 percent of the population, 42.5 years being the average age of onset[3].

Inflammation is thought to be a critical regulator in type-2 diabetes mellitus development. Interleukin-6(IL-6), a proinflammatory cytokine, has been linked to the development of insulin resistance and, as a result, to the pathophysiology of type-2 diabetes mellitus. Diagnosis of T2DM is delayed, which leads to an increase in microvascular and macro vascular disease.

Diabetic nephropathy (DN) is a prominent chronic microvascular consequence of T2DM and a main cause of kidney failure. One of the potential pathways for explaining microvascular problems in T2DM is inflammation. IL-6 is a versatile cytokine that plays a role in immunological and inflammatory responses. Diabetic nephropathy is one of the most common causes of chronic kidney failure worldwide[4]. Inflammation has a key role in the onset and progression of renal disease in diabetic patients[5].

AIMS AND OBJECTIVES : This study aimed to estimate serum IL-6 levels in patients of type-2 diabetes mellitus and to correlate serum IL-6 with diabetic nephropathy.

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METHODS : This hospital based observational study was conducted for 1 year i.e. from 1st September 2020 to 31st

August 2021. All diagnosed cases of type-2 diabetes mellitus as per guidelines by American Diabetic Association attending the Department of Medicine of Fakhruddin Ali Ahmed Medical college and hospital, Barpeta, Assam were screened and a total number of 60 cases with type-2 diabetes mellitus greater than or equal to 13 years of age irrespective of glucose control and treatment were included in this study. Sample size was chosen keeping in view the purpose and feasibility of the study since this study requires laboratory work. A group of 30 healthy controls were taken. Informed consent was taken from the patients and subjects taken up for the study. A detailed proforma was filled up for each patient including age, sex, hospital no., detailed history, and clinical examination done. Laboratory parameters and necessary investigation were done. This hospital based observational study was conducted for 1 year i.e. from 1st September 2020 to 31st August 2021. All diagnosed cases of type-2 diabetes mellitus as per guidelines by American Diabetic Association attending the Department of Medicine of Fakhruddin Ali Ahmed Medical college and hospital, Barpeta, Assam were screened and a total number of 60 cases with type-2 diabetes mellitus greater than or equal to 13 years of age irrespective of glucose control and treatment were included in this study. Sample size was chosen keeping in view the purpose and feasibility of the study since this study requires laboratory work. A group of 30 healthy controls were taken. Informed consent was taken from the patients and subjects taken up for the study. A detailed proforma was filled up for each patient including age, sex, hospital no., detailed history, and clinical examination done. Laboratory parameters and necessary investigation were done.

Estimation of IL-6 was done by quantitative detection in serum by enzyme linked immunosorbent assay (ELISA). An antihuman IL-6 coating antibody is adsorbed onto microwells. A biotin conjugated antihuman IL-6 antibody is added which binds to the human IL-6 captured by the first antibody. Following incubation, unbound biotin- conjugated antihuman IL-6 antibody is removed during a wash step. Streptavidin HRP is added and binds to the biotin conjugated antihuman IL-6 antibody. Following incubation, unbound streptavidin HRP is removed during a wash step and substrate solution reactive with HRP is added to the wells. A coloured product is formed in proportion to the amount of human IL-6 present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450nm primary wave length on a spectro-photometer. A standard curve is prepared from 7 human IL-6 standard dilutions and human IL-6 sample concentration is determined. Results must be read immediately after the stop solution is added or within an hour if microwell strips are stored at 2-8^{0}C in the dark.

RESULTS :

Out of the 60 subjects of case, i.e. patients with type-2 diabetes mellitus, there were 21(35%) females and 39(65%) males. And in the 30 subjects in control group, there were 10(33%) females and 20(67%) males. The mean age of the subjects in case group was 49.3+11.9 years and in control group was 52.4+14.2 years, both groups had no significant difference in their mean values.

The highest mean 24 hours urine albuminuria level 984.67+682.30 mg/day was noticed in macro albuminuria group and the lowest mean 24 hours urine albuminuria level 16.68±6.4 mg/day was noticed in normoalbuminuria group. There was significant (p <0.0001) difference between the groups. The highest mean eGFR level 99.45±25.42 mL/min/1/.73m2 was noticed in normoalbuminuria group and the lowest mean eGFR level 45±27.16mL/min/1/.73m2 was noticed in macro albuminuria group.

Table 1:Mean values of 24 hr Urinary Albumin Excretion(UAE) (mg/day) and eGFR (ml/min/1.73m2) in relation to urinary albumin

Mean±SD	NORMOALBUMINU-RIA	MICROALBUMIN-URIA	MACROALBUMINU-RIA
24 hr Urinary Albumin Excretion (mg/day)	16.68±6.4	112.9±62.1	984.67+682.30
eGFR (mL/min/1.73m2)	99.45±25.42	59.55±28.47	45±27.16

Table 2: Comparison of Interleukin-6 ,IL-6 values between case and control measured in pg/ml.

IL-6	Case	Control	p-value
Mean±SD	27.2±35.9	2.5±0.6	<0.0001

The mean IL-6 of the subjects in case group was 27.2pg/ml and in control group was 2.5 pg/ml, the case group had significantly higher (p<0.0001)mean value than the control group.

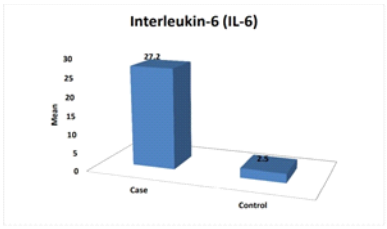


Table 3: Mean values of Interleukin-6 (IL-6) (pg/ml) in relation to urinary albumin excretion

The highest mean level of IL-6, 79.13±19.31 pg/ml was noticed in the macro albuminuria group and the lowest mean level of IL-6, 2.36±0.88 pg/ml was noticed in normoalbuminuria group. There was significant (p<0.0001) difference between the groups.

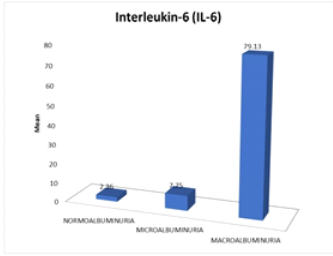
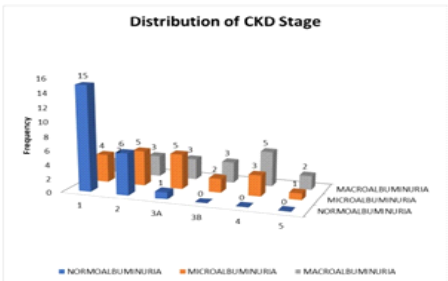


Table 4: Distribution of CKD Stage (according to eGFR) in relation to urinary albumin excretion

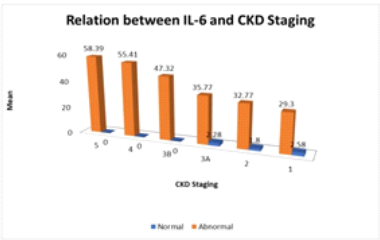
CKD Stage	NORMOALBUMINURIA		MICROALBUMINURIA		MACROALBUMINURIA		Total		p-value
	Count	%	Count	%	Count	%	Count	%	
1	15	68.18%	4	20.00%	2	11.11%	21	35.00%	0.0034
2	6	27.27%	5	25.00%	3	16.67%	14	23.33%	
3A	1	4.55%	5	25.00%	3	16.67%	9	15.00%	
3B	0	0.00%	2	10.00%	3	16.67%	5	8.33%	
4	0	0.00%	3	15.00%	5	27.78%	8	13.33%	
5	0	0.00%	1	5.00%	2	11.11%	3	5.00%	
Grand Total	22	100.00%	20	100.00%	18	100.00%	60	100.00%	



IL-6	NORMOALB-UMINURIA	MICROALBUMIN-URIA	MACROALBUM-INURIA	p-value
Mean±SD	2.36±0.88	7.75±1.17	79.13±19.31	<0.0001

Out of 22 subjects in normoalbuminuria group, 15 subjects were in CKD stage 1, 6 subjects were in CKD stage 2, 1 subject was in CKD stage 3A, none of them were in CKD stage 3B, 4 and 5. Out of 20 subjects in micro albuminuria group, 4 subjects were in CKD stage 1, 5 subjects each were in CKD stage 2 and CKD stage 3A, 2 subjects were in CKD stage3B, 3 subjects were in CKD stage 4, and 1 subject was in CKD stage 5.Out of 18 subjects in macro albuminuria group,2 subjects were in CKD stage 1, 3 subjects each were in CKD stage 2, stage 3A, and stage 3B, 5 subjects were in CKD stage 4, and 2 subjects were in CKD stage 5.There was significant (0.0034) difference between the groups.

IL-6	CKD Staging						pvalue
	1	2	3A	3B	4	5	
Mean ±SD	10.22± 20.09	19.5 ±31. 24	32.05± 40.39	47.32± 40.13	55.41±4 3.25	58.39± 46.45	0.0024



The mean values of CRP as per CKD stage were- 4.57+8.22 mg/dl, 8.38+10.03 mg/dl, 18.8+6.83mg/dl,26.9+8.45mg/dl,36+7.14mg/ dl, 46.53+12.11 mg/dl in the stage 1, 2, 3A, 3B, 4, and 5 respectively. There was significant positive correlation between the values of CRP and CKD stage , with the progression of different stages of nephropathy.

CONCLUSION :

In conclusion, the chief observation seen in our present study was that there is a significant difference between the inflammatory parameters IL-6 between diabetic and non diabetic groups, and also a significant relation between the inflammatory parameter and urinary albumin excretion as well as severity of diabetic nephropathy. The values of IL-6 showed positive correlation with urinary albumin excretion and a negative correlation with estimated GFR. A decrease in estimated GFR signifies a decrease in renal function, denoting severity of diabetic nephropathy. It indicates that inflammation may play a significant role in the pathogenetic mechanism of type-2 diabetes mellitus and its complications, one of them being diabetic nephropathy. Therefore, IL-6 estimation at earlier stages of type-2 diabetes mellitus can help to detect risk for nephropathy in the early phase and help prevent development and progression of diabetic nephropathy by taking appropriate measures at the earliest.

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