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Role of Inflammatory Markers in Development of Type 2 Diabetes Mellitus

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Abstract:- Diabetes is a condition that impairs the body's ability to process blood glucose. Type 2 diabetes has several causes- genetics and lifestyle are the most important ones. A combination of these factors can cause insulin resistance, when your body doesn't use insulin as well as it should. Insulin resistance is the most common cause of type 2 diabetes. Alcohol abuse is said to influence several changes in the cytokine levels. The present study was undertaken to evaluate the role of cytokines, which seems to be the probable cause of insulin resistance leading to type 2 diabetes. 400 subjects were included in the study divided into Control (Group I) 100 non-alcoholic control, 100 alcoholic healthy controls. Diabetic subjects as Group II- 100 newly diagnosed non-alcoholic diabetic patients and 100 newly diagnosed alcoholic diabetic patients respectively, both from the age group of 35-55 years. The result specified that there is a strong relation between increased cytokines levels due to alcohol abuse, insulin resistance and diabetic progression and its complication.

Keywords:- *Diabetes Mellitus, Interleukins, Cytokines, Alcohol, IL1, IL-6, IL-8, IL-10*

I. INTRODUCTION

Alcohol (ethanol) is known as a depressant drug. It is said that alcohol slows down the function of central nervous system. Heavy alcohol consumption is said to alter emotions, movements, vision, speech. Alcohol is also said to alter the cytokine levels in the body [1]. Cytokines are the substances such as interferon, interleukin, growth factors which are secreted by certain cells of the immune system. There are various physiological and behavior changes due to alcohol abuse leading to discrepancy in the inflammatory markers. [2]

Interleukins is a class of glycoproteins produced by leucocytes for regulating immune responses [3]. Inflammation and immunity are mediated through cytokines. Interleukins exert both inflammatory and anti-inflammatory actions [4]. According to the WHO, approx. 1.6 million death were caused due to diabetes in 2016 [5]. Type 2 diabetes (T2D) is the most common form of diabetes. Ineffective use of insulin by the body leads to type 2 diabetes (formerly known as non-insulin dependent). T2D is largely said to be caused due to excess body weight and physical inactivity [6]. This in turn displays the activation of chronic immune system in pancreatic islet, insulin insensitivity at the sites of diabetic complications.

Interleukin (IL)-1 (IL-1) mediates inflammatory functions, promotes activation, mediates co-stimulation responses and stimulates secretion of cytokines and other acute phase proteins [3]. IL-6 arbitrate inflammatory actions, induces proliferation and differentiation [7]. IL-8 is a chemokine produced by macrophages and other cell types such as epithelial cells, airway smooth muscle cells and endothelial cells [8]. Immune suppression is conciliated by IL-10, crucial part of IL-10 is inhibiting cytokine against the inflammation [9].

IL-1 is said to modulate the basic metabolic rate, blood pressure and blood glucose levels. IL-1 diminishes the insulin secretion and persuade beta cells apoptosis leading to T2D. IL-1 has a significant role in endocrinology and in regulation of responses related with inflammation [10]. The role of IL-1 in the process of inflammation is well established but mechanism by which it causes T2D still remains unclear.

IL-6 is a protein produced by various cells. It helps regulate immune responses, which makes the IL-6 test potentially useful as a marker of immune system activation. IL-6 can be elevated with inflammation, infection, autoimmune disorders, cardiovascular diseases, and some cancers. IL-6 plays a crucial role in the pathogenesis of T2D. IL-6 is present in almost all the cells, but its overproduction leads to impairment by phosphorylation of insulin substrate 1 and insulin receptor, which in turns induces the expression of suppressor of cytokine signaling 3 gene (SOCS-3) inhibiting the insulin signaling potentially thereby causing insulin resistance which finally leads to T2DM. (fig. 1)

L-8 is a chemo-attractant cytokine produced by a variety of tissues and blood cells. IL-8 is an important mediator of host response to injury, trauma and inflammation. IL-8 plays a role in activation of neutrophils,

neutrophil chemotactic factor, T-cells and basophils [11]. IL-8 promotes the macrophages infiltration in adipose tissue causing systemic inflammation leading to adipose tissue dysfunction. The systemic inflammation and impaired blood flow leads to insulin resistance and thereby T2D [7, 12] (Fig. 2).

Many evidences point towards the fact that immune responses are possibly the cause of inducing T2D. IL-10 here plays a central role as inhibitory cytokine against the action of inflammatory cytokine such as IL-12 [13].

The relationship between heavy alcohol consumption the elevation of the cytokines and inflammation causing the induction of T2DM has long been of interest, but there is a very scant literature about the mechanism involved in the progression of T2D. Hence this study was undertaken to see the possibly mechanism which can be of diagnostic and prognostic use in future for the clinicians.

II. MATERIALS AND METHODS

The following study was carried out in the Department of Biochemistry, Grant Govt. Medical College and Sir JJ Group of Hospitals, Mumbai where all the requirement facilities were available.

The present study included 400 subjects. Out of 400 subjects 200 were controls –non-alcoholic and alcoholic (group I) and 200 were subjects with newly diagnosed type 2 diabetes mellitus non-alcoholic and alcoholic (group II) respectively.

- *Inclusion & Exclusion Criteria-*

Subjects with T2D without any systemic disease and within the age group of 35- 55 years were included. Individuals with type 1 diabetes, chronic diseases like HIV, cancer, tuberculosis, kidney and liver diseases were excluded. Smokers smoking more than 5 cigarettes/bidis were also excluded.

Informed written consent was obtained from all individuals participants included in the study. All procedures performed in the study were in accordance with the ethical standards of the institution after the Institutional Ethical Committee approval.

- *Sample Collection-*

The subjects were given prior information regarding collection. The patients were instructed about 12 -13 hours of fasting before collection. 7ml of fasting blood sample was collected in a plain tube and kept for 30min for clotting. 2ml blood was collected in fluoride blub for glucose estimation (fasting and post-prandial). The samples were stored at 4°C if required. The collected/stored samples were later used for biochemical estimations of cytokines like IL-1, IL-6, IL-8 and IL-10 by Siemens Immulite 1000 Fully Automated Enzyme Amplified Chemiluminescent Immunoassay based analyzer with kits – it is a solid phase enzyme –labeled chemiluminescent immunometric assay. Glucose estimation was done using GOD-POD method colorimetrically [14].

- *Statistical Analysis-*

Statistical analysis was done using students 't' test. The values obtained were tabulated and mean and standard deviation (SD) of all the parameters were calculated. The test of significance applied was ANOVA (Analysis of Variance) for comparison. p value $\leq$ 0.001 was considered as statistically significant.

III. RESULT AND DISCUSSION

The present study was undertaken to understand if the newly diagnosed non-alcoholic T2D patients and alcoholic T2D patients resulted in changes in cytokines levels and its rate of inflammation. The results indicated that there is a considerable increase in the most of the cytokines levels of T2D patients as compared to healthy controls. Also, it was noted that heavy alcohol intake alters the concentrations of the certain cytokines.

The study unwinds the possible role and relative mechanism of the cytokines and induction of T2D. High significance of cytokines were observed in DM patients ($p<0.0001$). Remarkable high levels of IL-10 was noted when compared to the other cytokines (IL-1, 6 & 8) (Graph 1). High levels of IL-1 was marked when compared to IL-6 and IL-8 (Graph 1). Diabetic group showed marked elevation of circulating IL-8 levels. Cytokines levels were highly significant in DM. Whereas, remarkable low levels of IL-10 were noted in DM (table 1, Graph 2). Among group II the newly diagnosed alcoholic diabetic patients showed significant decreased in IL-10 levels when compared with non-alcoholic newly diagnosed diabetic subjects.

The cytokines are seen to be an important factor mediating the systemic inflammation causing dysfunction of certain cytokine producing cells, adipose cells etc. leading to insulin resistance due to certain pathophysiological changes (fig. 1 & 2) thereby causing T2D. The alcohol is said to trigger the severe alteration in the concentration of the different cytokines.

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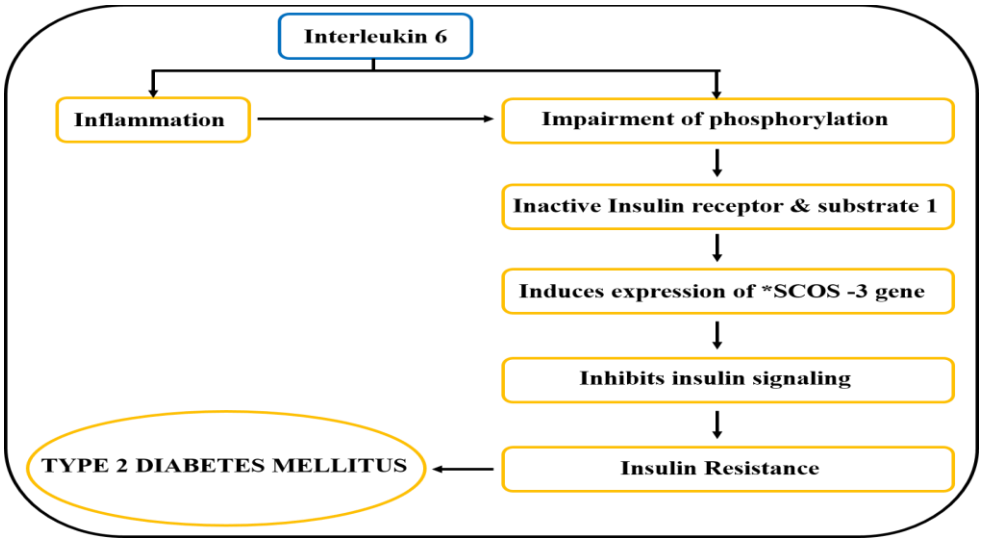

Fig 1:- Pathophysiology of IL-6

The heavy consumption of alcohol increases inflammation by persuading the release of proinflammatory cytokines i.e. IL-6. In response to this inflammation the immune system releases IL-10 as a immunosuppressive. IL-10 hyporesponsiveness may said to contribute chronic inflammation in T2D. IL-10 hyporesponsiveness may hold therapeutic potential for reducing inflammation in T2D. Also, the other interleukins (IL-1, IL-6 and IL-8) are said to bestow major share in the inflammation in T2D. Several studies exhibit that in alcoholic diabetic patients there is increase seen in the proinflammatory cytokines (IL-6, IL-10) [15, 16, 17]. Our study correlation with Barry and associates who stated that IL-10 played an important and defensive role in T2D by inhibiting the inflammatory effect caused by the release of certain cytokines [13].

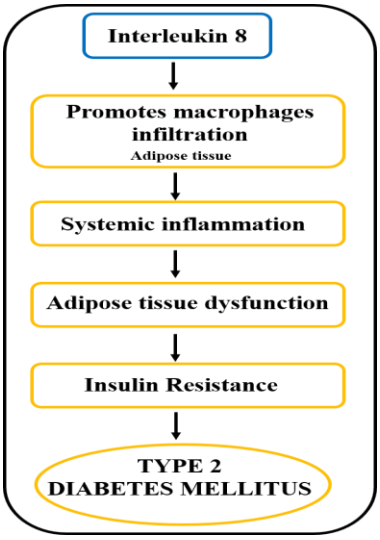

Fig 2:- Pathophysiology of IL-8

Risk factors	Group I- Control		Group II- Newly diagnosed Diabetics	
	Non-alcoholic	Alcoholic	Non-alcoholic	Alcoholic
Cytokines -				
Interleukin 1	2.01 ± 0.21	2.10 ± 0.25	2.81 ± 0.31*	2.93 ± 0.38*
Interleukin 6	0.25 ± 0.06	0.3 ± 0.07	0.55 ± 0.14*	0.65 ± 0.2*
Interleukin 8	0.73 ± 0.39	0.8 ± 0.41	1.52 ±0.50*	1.86 ± 0.58*
Interleukin 10	16.11 ± 2.27	16.32 ± 2.41	9.53 ± 2.27*	8.01 ± 2.01*
Blood Glucose	83.26 ± 4.8	92 ± 5.1	174.4 ± 9.6*	192.67 ± 10.73*

Table 1:- Cytokines (ng/ml) and Blood Glucose (mg/dl) ([#])
[#] Data reported as mean ± SD

Paired t test: *p<0.0001 compared to Group II (Newly diagnosed)
Unpaired t test: [#]p<0.0001 Compared to Group I (Control)
Unpaired t test: [$] p<0.0001 Compared to Group I (Control)

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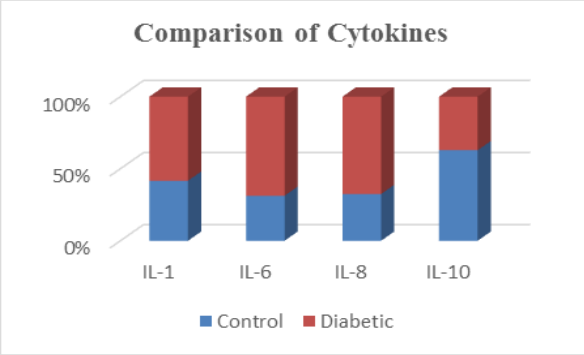
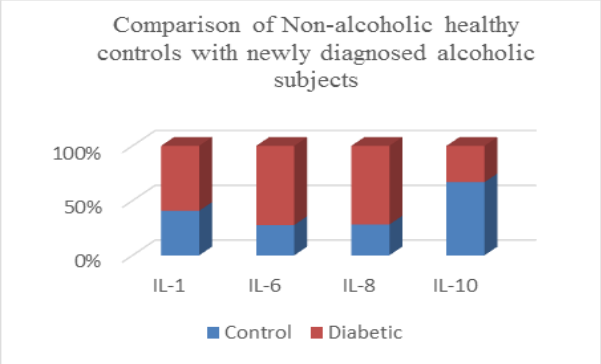


Graph 1



Graph 2

IV. CONCLUSION

The study signifies that cytokines plays a curial role through various mechanisms, some at tissue level or gene level which directly or indirectly is responsible for cell dysfunction thereby provoking insulin resistance due to which the body fails to respond to the hormone insulin. Insulin resistance is the constraining factor leading to T2D, prediabetes etc. Alcohol abuse being a disrupting factor for the mediating release of many inflammatory cytokines, thereby intensifying the inflammatory condition.

Potential studies are further required to assess the felicitous comparison of cytokines in the progression of T2D and to elucidate the certainty that cytokines can be a useful tool and a novel marker for risk stratification in T2D patients.

Additionally studies of cytokine inhibitors could be of great value which may play a major role in the prevention of diabetic progression and its complication.

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