THE ROLE OF TNF-ALPHA AND IL-6 LEVEL WITH IR IN TYPE-II DIABETES MELLITUS.

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ABSTRACT **Objective :** Diabetes mellitus is also known as inflammatory disease because persistent hyperglycemia may stimulate the innate immune system in body. This study is mainly focusing on the possible role of proinflammatory markers TNF-α and interleukin-6 along with insulin resistance in type -2 diabetic subjects.

Material & Methods : The study was conducted in 200 human subjects out of whom 100 were normal healthy individuals (group I) and 100 were type 2 diabetic subjects with complication (group II).

RESULTS : Showing the comparative changes of immunological and insulin resistance (HOMA-IR) parameters in group I healthy male and female subjects and group II diabetic male and female subjects. TNF-α, IL-6,(HOMA-IR) were highly significant at (P<0.001).

CONLUSION: Outcome of this study showed that IL-6 and TNF-α plays an important role in insulin resistance. The adipokines produced by adipocytes or by adipose tissue infiltrating macrophages, are able to induce a low grade inflammation state that could play a central role in obesity and type-2 diabetes related insulin resistance.

Introduction : Diabetes mellitus, a common metabolic disorder resulting from defect in insulin secretion or action or both and characterized by an increase in insulin resistance in conjugation with the inability of pancreatic beta cells to secrete sufficient insulin to compensate(1). The inhibition of signaling downstream of the insulin receptor is a primary mechanism through which inflammatory signaling leads to insulin resistance. Insulin resistance and insulin deficiency give rise to a hyperglycemic state that is a major risk factor for the development of diabetic and its complications (2). A close connection between insulin resistance and classic inflammatory signalling pathways has also recently been identified (3). Recently it has become clear that inflammatory signalling pathways can also become activated by metabolic stresses originating from inside the cell as well as by extracellular signalling molecules. It has been demonstrated that obesity overloads the functional capacity of the ER and that this ER stress leads to the activation of inflammatory signalling pathways and thus contributes to insulin resistance (4,5,6,). Insulin resistance is the driving force of hyperglycemia of type- 2 diabetes (7). Several studies have demonstrated elevated levels of TNF-α and IL-6 among individuals with insulin resistance (8,9). TNF-α plays a direct pathogenic role in glucose metabolism. Impaired insulin sensitivity in skeletal muscle is a major feature of type-2 diabetes (10). Proinflammatory cytokines, IL-6 is one of several proinflammatory cytokines that have been associated with insulin resistance and type-2 diabetes (11).

Method and Material : The study was conducted in 200 human subjects out of whom 100 were normal healthy individuals (group I) and 100 were type 2 diabetic subjects with complication (group II). Age matched healthy control subjects were selected from known families. The written consent of patients was also taken before starting the study. A record of clinical history and previous investigations of patients disorders were compiled in a Performa (Performa enclosed). A Performa containing the relevant findings of clinical, biochemical and physiological investigations were recorded on preset questionnaire as base line record. All ethical measures were taken prior and during the study.

5 ml of blood sample was withdrawn from the anticubital vein following overnight fasting. The blood sample was collected in plain, fluoride and EDTA vacutainers. The blood sample was analyzed for biochemical and immunological investigations.

Immunological markersinterleukin-6 and tumour necrosis factor- were estimated by a highly sensitive sand witch-enzyme linked immunosorbent assay (ELISA) method in a commercially available kit (Immunotech, Backman Coulter, France). The assay was performed exactly as recommended by the manufacturer. Data analysis was performed by using SPSS software version 16.0 by one ways ANNOVA utilizing Dunnet T3 test.

Result and observation

Table 1 and Graph 1 Showing the comparative changes of immunological and insulin resistance (HOMA-IR) parameters in group I healthy male subjects (N=70) and group II (N=52) diabetic male subjects. TNF-α, IL-6, (HOMA-IR) were highly significant at (P<0.001) in group II subjects.

Table 2 and graph 2 Showing the comparative changes of immunological and insulin resistance (H0MA-IR) parameters in group I healthy female subjects (N=30) and group II diabetic female subjects (N=48). TNF-α, IL-6,and HOMA-IR were highly significant at (P<0.001) in Group II subjects.
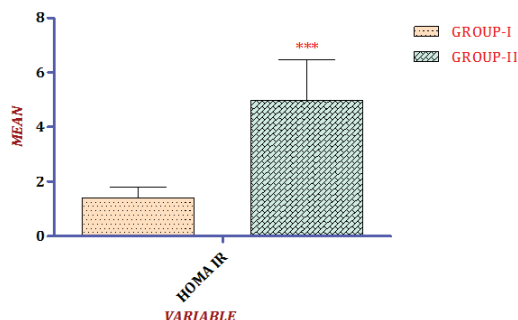
Graph 1 - Showing the comparative change in HOMA-IR in group I and II male subjects
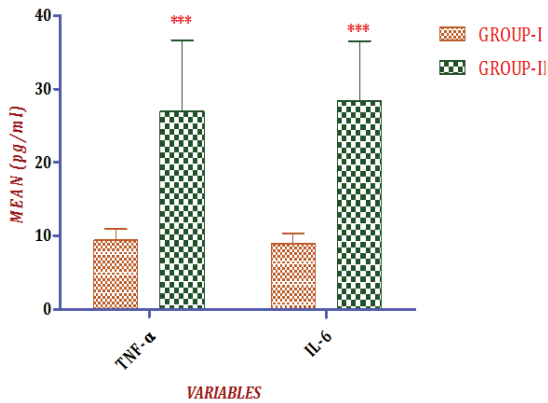
Graph - 2 showing the comparative changes of immunological-parameter in group I and II male subjects

Table no.1 showing comparative changes of biochemical and immunological parameters in Group I and Group II Male subjects.

GROUPS		HOMA IR	TNF-α	IL-6
GROUP I MALE SUBJECTS (n = 52)	Min	00.64	07.10	05.60
	Max	02.69	18.00	11.30
	Mean	01.41	09.37	08.91
	± SD	00.38	01.58	01.37
GROUP II MALE SUBJECTS (n = 70)	Min	2.14	15.00	12.00
	Max	8.51	56.00	48.60
	Mean	04.96	26.93	28.38
	± SD	01.50***	09.74***	08.17***

*** Highly Significant at 0.001 (p<0.001) between Group I and Group II Male

Table no.2 Showing comparative changes of biochemical and immunological parameters in Group I and Group II Female subjects.

GROUPS		HOMA IR	TNF-α	IL-6
GROUP I FEMALE SUBJECTS (n =48)	Min	01.15	6.00	05.60
	Max	03.43	15.00	13.40
	Mean	02.12	9.17	08.98
	± SD	00.74	01.44	01.59
GROUP- II FEMALE SUBJECTS (n = 30)	Min	01.82	11.00	14.00
	Max	08.66	53.09	39.50
	Mean	04.79	28.63	24.81
	± SD	01.39***	11.53***	06.65***

*** Highly Significant at 0.001 (p<0.001) between Group I and Group II Female

Discussion : According to many studies, Diabetes mellitus is also known as inflammatory disease because persistent hyperglycemia may stimulate the innate immune system in body. Skeletal muscle possesses many components of the innate immune system, including cytokine receptors and toll like receptor (TLRs) (12,13,14) suggested that long term innate immune system activation along with genetic and environmental factors in chronic inflammation elicits disease instead of repair, leading to the development of type-2 DM. In newly diagnosed or established patients with type-2 DM have shown that acute phase reactants and proinflammatory cytokines are positively correlated with insulin resistance (15,16). The other study parameters in diabetic male and female subjects of group II and group III showed same pattern of significant change in urea, creatinine and insulin resistance as compared to group I (P< 0.01) while highly significant (P< 0.001) increase of inflammatory cytokine TNF-α and IL-6 were observed. These findings are consistent with the research work (17,18,19,20,21)

This study is mainly focusing on the possible role of proinflammatory markers TNF-α and interleukin-6 along with insulin resistance in type -2 diabetic subjects.

Several interesting observations provide further evidence that IL-6 plays an important role in insulin resistance. The predominant site of IL-6 production has until recently been thought to be macrophages and peripheral mononuclear cells. However, recent evidence suggests that adipose tissue has been shown to produce 10-35 % of IL-6 in a resting individuals and this production increases with increase adiposity (22). linked to inhibition of hepatic glycogen synthase, activation of glycogen phosphorylase and lipolysis and increase triglyceride production (23,24). As a result of these observations, it has been hypothesized that IL-6 plays a role as a glucoregulatory hormone.

TNF-α is another proinflammatory cytokine which was observed extensively in this study. It is produced by a variety of cell types, but mostly by macrophages and lymphocytes. After observing the TNF-α calculation, we found a significant increase among group III as compared to group I with P-value (< 0.001) as illustrated in (Table 1-2) (Graph 1, 2, 3) is thought to play a major role in the Pathophysiology of insulin resistance through the phosphorylation of the insulin receptor substrate-1(IRS-1) protein on serine residues.

This could prevent its interaction with the insulin receptor beta sub unit and stop the insulin signaling pathway (25).

Insulin resistance is a fundamental defect that precedes the development of the full insulin resistance syndrome as well as β cell failure and type-2 diabetes mellitus. TNF-α a paracrine/autocrine factor highly expressed in adipose tissue of obese humans subjects and are implicated in the induction of insulin resistance seen in obesity and type-2 diabetes (26). Several studies have documented increased adipose expression of TNF-α mRNA in non-diabetic subjects with obesity dependent insulin resistance, in normoglycemic subjects with increased insulin resistance and in type-2 diabetic subjects (27).

It is possible that in coming years the hope of new therapeutic strategies based on anti-inflammatory properties with beneficial actions on diabetic complications can be translated in to real clinical treatments.

Conclusion : The pathogenetic vision of diabetes mellitus has changed in the last few years, where inflammatory pathways playing pivotal roles in the development and progression of diabetes complications. These new pathogenic factors lead to a consideration of new therapeutic approaches. Modulation of inflammatory processes in the setting of diabetes is now days a matter of great interest.

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