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INSULIN RESISTANCE ALTER THE LEVELS OF IL-4, IL-5 AND IL-13 IN TYPE 2 DIABETES MELLITUS WITH LESS THAN 5 YEARS OF DURATION

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ABSTRACT The harmonious biochemical processes in a healthy individual are due to the balance between essential molecules that are necessary for the maintenance of physiology of the living system. These include energy bio-molecules, co-factors for oxidation to attain energy expenditure, regulatory hormones, and protective immune system. **Objectives:** Identifying predictive factors for 2DM will be beneficial to developing effective prevention and early detection of the disease. Therefore, the novelty of this study is to evaluate whether inflammatory markers are different in individuals with versus without T2DM. To assess the effect of glucose, insulin, Homeostasis metabolic assessment-estimated insulin resistance (HOMA-IR), lipids on cytokine variables in type 2DM subjects. **Materials& Methods:** Two hundred and thirty individuals are recruited in to this present study after the approval from Institutional ethical committee for the present study. The study was conducted in the Department of Biochemistry, Malwanchal university, Indore, India. Age & sex matched one hundred and fifteen human non-2DM individuals were taken into healthy control group. One hundred and fifteen subjects, on treatment for 2DM were included in second group.**Results:** On comparison of age, Tc, and Ldl parameters between 2DM and control group subjects showed insignificant differences, whereas parameters of FBS ($t=12.24$; $df=228$; $P<0.001$) HbA1c ($t=16.40$; $df=228$; $P<0.05$) and insulin mean levels ($t=2.70$; $df=228$; $P<0.05$) showed a significant difference. Decrease in the levels of serum IL-4 was observed in 2DM subjects when compared with healthy control subjects. On the contrary, we observed increase in the values of IL-5 and IL-13 in 2DM subjects when compared with healthy control subjects. In case of IL-10 and IL-12 values, we observed no significance when compared between the group subjects. **Conclusion:** The present study concludes that there is association of IL-4, IL-5 and IL-13 with relation to 2DM. These cytokines can be used as biomarkers for early identification and diagnosis of secondary complications in 2DM individuals.

KEYWORDS : Interleukins, Insulin resistance, Cytokines, Hormones

INTRODUCTION:

The harmonious biochemical processes in a healthy individual are due to the balance between essential molecules that are necessary for the maintenance of physiology of the living system [1,2]. These include energy bio-molecules, co-factors for oxidation to attain energy expenditure, regulatory hormones, and protective immune system [3]. Apparently through evolution, biological cell has developed innate dynamic processes vital to safeguard the obnoxious effects of the external environmental forces and also from obnoxious internal forces [1-3]. Occurrence or production of any molecule in excess would proceed to dys-balance leading to deposition of products or adducts and excitation of unwanted responders [4,5].

According to World Health Organization report, around 1.6 million die of diabetic complications in the world every year [6,7]. The prevalence of hypertension is approximately about 70% in 2DM so that one-fourth to three-fourths of the secondary complications including cardiovascular and renal of 2DM are attributed to hypertension [6,7]. The recently concluded Trials and studies have reported that intensive insulin therapy has been shown to reduce the development of mico- and macro-vascular complications in Type 1 diabetes mellitus patients [8-12]. These studies have also shown that bringing the glucose levels to near physiological levels can reduce the morbidity of hypertension, diabetic retinopathy and diabetic nephropathy [8-12].

Insulin Resistance (IR), hyperglycemia, dyslipidemia and inflammation are the risk factors that can initiate the development of hypertension in Type 2 diabetes mellitus (2DM) [13]. In 2DM, hyperglycemia drives non-enzymatic glycation of proteins and lipids leading to the formation of Advanced Glycation of End products (AGEs) including glycated Hemoglobin (HbA1C) and oxidized Low Density Lipoproteins cholesterol (oxLDLc) [14]. Studies across the globe suggested that glycated lipid namely oxLDLc bind to the vessel walls via a specific receptor, which in turn induces the development of atheromatous plaques [10]. Similarly, AGEs also attaches to the vessel walls through receptor and influence to initiate cytokine stress [15]. Cytokines are glycoprotein molecules released by the immune cells when they come into contact with the altered glycated molecules [16,17]. Cytokines, thus expressed play in immune response as either pro-inflammatory or anti-inflammatory [15-17]. These steps increase in inflammation that produces tissue damage which may trigger an impending mechanism for development of diabetic complications [18].

Identifying predictive factors for 2DM will be beneficial to developing effective prevention and early detection of the disease. The role of cytokines in 2DM individuals has not yet been well clarified, especially in this specific part of India (Indore region). Moreover, there has been little study on 2DM individuals in the initial period of the disease and during the course of disease on blood glucose and cytokine levels as a damaging factor have not been considered in existing studies, Therefore, the novelty of this study is to evaluate whether anti-inflammatory and pro-inflammatory markers are different in individuals with versus without 2DM. To assess the effect of glucose, insulin, Homeostasis metabolic assessment-estimated insulin resistance (HOMA-IR), lipids on cytokine variables in type 2DM subjects.

MATERIALS & METHODS:

Two hundred and thirty individuals are recruited in to this present study after the approval from Institutional ethical committee for the present study. The study was conducted in the Department of Biochemistry, Malwanchal university, Indore, India. Age & sex matched one hundred and fifteen human non-2DM individuals were taken into healthy control group. One hundred and fifteen subjects, on treatment for 2DM were included in second group. The diagnosis of 2DM was made according to the norms laid by American Diabetes Association. The diagnosis of 2DM group subjects was done by the consultants of Medicine department of Malwanchal university. Exclusion criteria were 2DM individuals, more than five years of known duration of 2DM, and with known complications. Inclusion criteria for healthy controls were non-diabetic, not taking

supplementations, and having no other complications. Five mL of fasting venous blood were drawn into fluoride and plane vials, after informed written consent from all the study group subjects with a disposable syringe & needle, under all aseptic conditions. Plasma and serum was separated by centrifuging the blood at 3000 rpm for 20 minutes. Samples were stored in aliquots at -20° C until assayed. Plasma glucose was estimated by using the method Glucose Oxidase and Peroxidase (DPEC – GOD/POD) purchased from Avantor laboratories. HbA1C was estimated by using the ClinRep complete kit on the BioRad HbA1c analyzers Diamat and Variant. Added 5 µl venous blood to 1.25 ml hemolysis reagent. Incubated hemolysate for 300 min at 37 C. Injected 20 µl into the HbA1c variant instrument and noted the percentage of glycated hemoglobin. Serum TC was estimated by using the method of Cholesterol Oxidase and Peroxidase (CHOD/POD) purchased from Transasia Biomedicals. The reagents were prepared according to the instructions provided in the kit manual. Serum Low density lipoprotein cholesterol was estimated with Friedewald's formula. Serum cytokines were estimated with multi-analyte Elisarray kit bought from Qiagen laboratories.

Statistical Analysis:
Microsoft excel was used to perform statistical analysis. Unpaired 't' test was performed to compare the means of variables between two groups. Percentages were also calculated. P <0.05 was considered significant.

RESULTS:

Biochemical Parameters in 2DM and Healthy control:
In the present study, the authors estimated age, fasting blood glucose (FBS) in plasma, serum Total cholesterol (Tc), Low density lipoproteins (Ldl), Glycated Hemoglobin (HbA1c), insulin, IL-4, Il-5, IL-10, IL-12 and IL-13 in both the group subjects. In addition to know the intensity of IR in both the group subjects, we calculated the HOMA-IR.

In Table 1, on comparison of age, Tc, and Ldl parameters between 2DM and control group subjects showed insignificant differences, whereas parameters of FBS (t=12.24; df=228; P<0.001) HbA1c (t=16.40; df=228; P<0.05) and insulin mean levels (t=2.70; df=228; P<0.05) showed a significant difference. Though the Ldl mean level of 2DM group subjects was on the higher side but when compared between the two groups, we observed approximately 40 percent high in 2DM individuals than the control. In case of HOMA-IR we observed almost fifty percent increase in level in 2DM subjects than in control subjects. We measured the percentages increase due to the fact that we included age and sex matched individuals in both the groups.

In figure 1, we observed statistical difference in the serum levels of IL-4, Il-5 and IL-13 when compared between 2DM group subjects and control group subjects. Decrease in the levels of serum IL-4 was observed in 2DM subjects when compared with healthy control subjects. On the contrary, we observed increase in the values of IL-5 and IL-13 in 2DM subjects when compared with healthy control subjects. In case of IL-10 and IL-12 values, we observed no significance when compared between the group subjects.

In figure 2, we observed a stable decline, with a negative regression of y = -0.248x + 38.06 when compared between HOMA-IR and IL-4 in 2DM students. Similar negative decline in regression (y = -0.053x + 2.67) we observed when HOMA-IR compared with IL-5 in healthy subjects (Fig. 4). In another, an incline depicted a positive regression (y = 0.007x + 15.76) when HOMA-IR of 2DM subjects was compared with serum IL-13. These regression analyses have shown the association that IR has an effect on the development of inflammation in type 2DM individuals.

DISCUSSION:

Objectives that are framed in this study are to evaluate whether inflammatory markers are different in individuals with versus without T2DM. For this to understand we assessed the effect of glucose, insulin, HOMA-IR, lipids on cytokine variables in type 2DM subjects. The 2DM group subjects showed altered FBS, HbA1c, and insulin when compared with healthy control group subjects. Insignificant difference was observed in the age parameter when compared between the two groups. In the present study pertaining to 2DM group subjects, an increase in FBS was observed, but not in healthy controls. This finding suggests that increased blood sugar in T2DM group may be caused by increase age in the subjects [10,11,13]. Studies related to 2DM have revealed that 2DM is one of the aging diseases in the present world and our finding also infers the concerns with age in T2DM subjects [19,20]. Studies have also reported that individuals above 40 years of age are more susceptible to develop T2DM [10,11]. Interesting point is that no correlation was observed in the control group when compared between age and blood sugar, however, we observed insignificant difference in age when compared between T2DM and controls. Keeping in view of this finding, suggests that people who have predisposition to T2DM, are prominently driven towards the initiation of the disease rather than the people who have little predisposition. Moreover, because older adults have the highest prevalence of diabetes, such individuals have traditionally not been included in some studies that involve research on diabetes [13,19,20].

It is interesting that a correlation between age and HbA1c was negative in the T2DM group and also control group. At first it seems contradictory, but possible explanation could be that the oxidative stress increases with age and increase in HbA1c is compensatory to the increase in age and the free radical production. Many studies have shown that people with T2DM tend to have higher oxidative stress compared to healthy controls compared with same age group individuals [21,22]. Though the present did not estimate free radicals but it is clear through the literature that oxidative stress is increased in T2DM patients and also in aged controls [19-22].

We observed significant difference in the serum levels of IL-4, Il-5 and IL-13 when compared between 2DM group subjects and control group subjects. Decrease in the levels of serum IL-4 was observed in 2DM subjects when compared with healthy control subjects. Moreover we observed negative association with HOMA-IR when compared with IL-4 in 2DM subjects. The lower level was due to hyperglycemia and HOMA-IR present in 2DM subjects. In some reports it has been reported that IL-4 has the ability to reduce IR and also immune response through improvement of anti-atherogenic properties [23,24]. Badr et al, in his report, demonstrated significant decrease in the IL-4 expression in 2DM subjects [25]. On the contrary, Tripathi et al has reported elevated levels of IL-4 gene expression in 2DM individuals when compared with healthy controls [26]. Whereas, in a report by Maier et al, has observed no association between IL-4 and susceptibility to 2DM [27]. It is imperative from the present study that, HOMA-IR and hyperglycemia are the factors for the decreased IL-4 production in 2DM subjects than healthy controls.

On the contrary, we observed increase in the values of IL-5 and IL-13 in 2DM subjects when compared with healthy control subjects. In case of IL-10 and IL-12 values, we observed no significance when compared between the group subjects. Similar negative decline in regression, we observed when HOMA-IR compared with IL-5 in healthy subjects. At first it seems contradictory, but possible inference could be that the increase in IL-5 is compensatory to the increase in HOMA-IR in healthy controls that can occur due to increase in age. In one study it has been reported that IL-5 levels are directly proportional to the oxLdl in patients affected with 2DM [28]. Apart from few studies, little research is known in relation to IL5 and 2DM around the world.

In another, an incline depicted a positive regression when HOMA-IR of 2DM subjects was compared with serum IL-13. These regression analyses have shown the association that IR has an effect on the development of inflammation in type 2DM individuals. The results in the present study, show that increase in the levels of IL-13 is could be due to the HOMA-IR. In one study, IL-13 levels were increased in tuberculosis patients suffering with 2DM [29]. In a study conducted on altered chemokine levels in individuals at risk of type 1 diabetes mellitus did not observe any significant difference in IL-13 in the project individuals [30]. In another report by Kreotowski et al., demonstrate that IL-13 were significantly high in patients who were diagnosed recently with DM [31]. Study by Al-Hamoudi et al [32], suggested increase in IL-13 levels in patients using electronic cigarettes that show inflammation in the oral cavity. In a study by Zhang et al [33]., reported higher levels of Il-13 in patients with clinical periodontal parameters of middle-aged and elderly patients with 2DM.

CONCLUSION:

Our study concludes that pro- and anti-inflammatory cytokines are responsible for the initiation of secondary complications in 2DM. The present study also concludes that there is association of IL-4, IL-5 and

IL-13 with relation to 2DM. These cytokines can be used as biomarkers for early identification and diagnosis of secondary complications in 2DM individuals.

Table 1: The mean values of different parameters of 2DM subjects and control subjects of the study

Variable	2DM Subjects (n=115)	Healthy Controls (n=115)
AGE (Years)	51.4±7.3	50.6±4.8
FBS (mg/dL)	132.8±45	86.6±11
PPBS (mg/dL)	195.9±62.2	123.7±11.5
HbA1C (gm%)	7.9±1.5	5.3±0.8
Insulin (µU/mL)	20.1±4.9	17.4±9.5
HOMA-IR	5.7±2.8	23.8±1.2
Total Cholesterol (mg/dL)	164.7±28.1	147.5±14.7
Low density lipoprotein (mg/dL)	149.1±53.6	128.8±21.4
IL-4 (pg/mL)	40.1±10.8	44.3±14.4
IL-5 (pg/mL)	15.9±1.0	12.3±0.5
IL-10 (pg/mL)	24.1±2.3	23.0±8.6
IL-12 (pg/mL)	146.8±43.8	153.3±12.0
IL-13 (pg/mL)	79.8±41.8	62.8±10.7

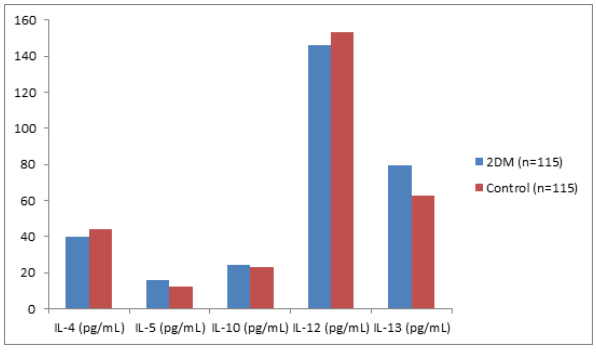

Figure 1: The mean values of IL-4, IL-5, IL-10, IL-12 and IL-13 in 2DM subjects and in healthy control subjects

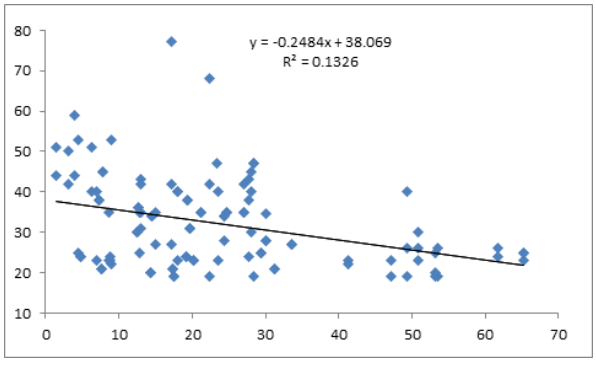

Figure 2: Scatter diagram showing relationship between HOMA-IR and IL-4 in 2DM subjects

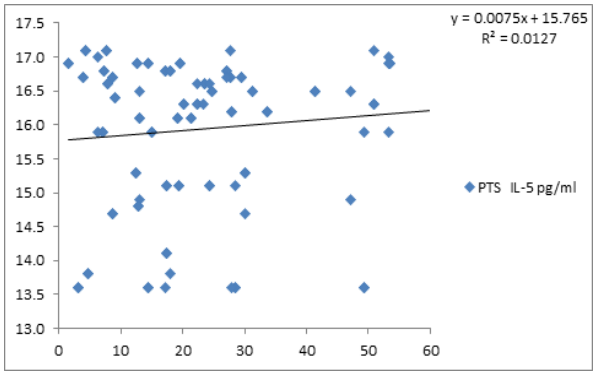

Figure 3: Scatter diagram showing relationship between HOMA-IR and IL-13 in 2DM subjects

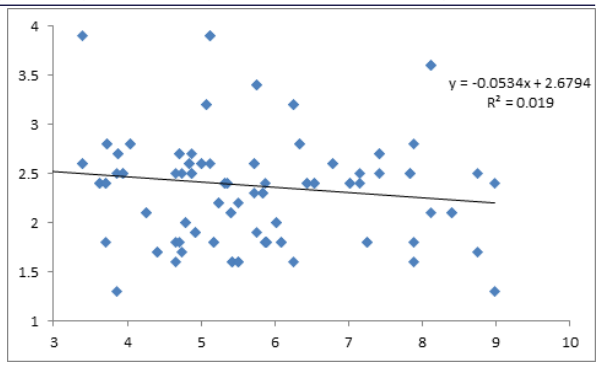

Figure 4: Scatter diagram showing relationship between HOMA-IR and IL-5 in healthy subjects

Conflict of interest:
None declared.

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