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Association of TNF-α with Fasting Glucose, Insulin and Insulin Resistance in Complete Glycemic Spectrum

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ABSTRACT

Background: The aim of the present study is to assess fasting glucose, fasting insulin, insulin resistance, and inflammation in complete glycemic spectrum and to study the association between them if any.

Materials and Method: Participants (30-50 years) of either gender were enrolled. Based on their family history of diabetes and glucose levels, they were grouped into normoglycemic non-first-degree relatives of diabetes, normoglycemic first degree relatives of diabetes, Prediabetes and diabetes. Fasting Glucose, Fasting insulin and Tumor necrosis α (TNF-α) concentrations were analyzed. Groups were compared using one-way ANOVA with LSD posthoc analysis. Correlation between the parameters were done using Pearson's correlation and linear regression analysis.

Results: We observed that fasting insulin, fasting glucose, TNF-α, and HOMA2 IR gradually increased as we moved along the glycemic spectrum from control, FDRD, prediabetes to diabetes, while HOMA2%S gradually decreased. HOMA2%B - there is an increase in FDRD as compared to controls, but it decreased in prediabetes and diabetes as compared to FDRD or controls. There was positive correlation between TNF-α and fasting glucose across the glycemic spectrum and no correlation with fasting insulin or insulin resistance.

Conclusion: Inflammation begins even in first degree relatives of diabetes and increases along with glucose levels along the glycemic spectrum.

Keywords: *First degree relatives of diabetes, prediabetes, HOMA2%B, HOMA2%S, HOMA2IR, HOMA-IR*

INTRODUCTION

Diabetes is increasing worldwide; Insulin resistance plays a significant role in the development of diabetes. Insulin resistance also leads to obesity, hypertension, dyslipidemia and cardiovascular diseases[(1)]. Hence, it requires earlier attention. In addition, to this, diabetes subjects display increased levels of inflammatory markers[(2)]. The underlying pathophysiology of diabetes development involves inflammation, which has been suggested by observing low-grade inflammation in subjects before developing diabetes[(3)]. One study documented the role of inflammatory markers in predicting the development of diabetes[(4).] TNF-α is one of the major inflammatory markers, produced by various cells such as, macrophages, T cells, neutrophils and monocytes. Moreover, exaggerated expression of TNF- α is associated with obesity related insulin resistance[(5)]. TNF-α causes metabolic derangements via various mechanisms - down regulation of genes involved in normal insulin action, targeting insulin signaling, inducing lipolysis and derangements of PPARγ, insulin-sensitizing nuclear receptor [(6)]. Few studies have narrated the potential role of TNF-α

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causing insulin resistance [7-9]. Increased levels of TNF-α has been documented in impaired glucose tolerance subjects [10, 11] whereas, some studies have not found any association [12]. Further, contradictory reports regarding the association of inflammatory markers with insulin resistance in first degree relatives of diabetes (FDRD) [13, 14] shows that the role of inflammatory markers causing insulin resistance is still inconclusive. Even though, studies have reported, the association of TNF- α with insulin resistance in diabetes [15, 16] and prediabetes [17], no studies have attempted to assess the role of TNF- α with insulin resistance in complete glycemic spectrum. Therefore, in the present study we aimed to assess the association of TNF- α with insulin resistance across the glycemic spectrum.

MATERIALS METHOD

This cross-sectional comparative study was conducted in Department of Physiology, JIPMER, Puducherry. Approval from institutes scientific and ethics committee was obtained for the study protocol. 160 participants in the age group of 30-50 years of either gender were enrolled for our study. Based on their family history of diabetes and glucose levels, obtained by history and oral glucose tolerance test respectively, they were grouped into normoglycemic non-first-degree relatives of diabetes (n=40), normoglycemic first degree relatives of diabetes (n=40), Prediabetes (n=40) and diabetes on oral hypoglycemic drugs (n=40). Subjects with organic disease, morbid obesity, hypertension and smokers were excluded from this study.

Biochemical markers: The fasting and postprandial blood glucose was estimated by glucose oxidase-peroxidase method (Genuine Biosystem). Fasting insulin and TNF-α were measured in plasma that had been drawn after an overnight fast and frozen at −80°C until assayed. Fasting insulin (DIAsource, Belgium) and TNF-α (Diaclone, France) concentrations were measured by enzyme-linked immunosorbent assay according to manufacturer guidelines.

We used the standalone version of the Excel spreadsheet implementation of the Homeostatic model assessment calculator - HOMA **Calculator** ©The University of Oxford 2013; The calculator uses the HOMA2 model that provides insulin sensitivity (HOMA2%S) and beta cell function (HOMA2%B) as percentage, where 100% is normal. This updated model accounts for variations in peripheral glucose and hepatic resistance and considers renal glucose loss too [18]. Hence can be used in hyperglycemic subjects and in subjects with high insulin section [19].

Statistical analysis: Comparisons of data across the groups were done using One-way ANOVA followed by post-hoc analysis using least significant difference (LSD). The statistically significance was set at $p<0.05$. Correlation between TNF- α and glucose, insulin, and derived insulin indices was done using Pearson's correlation and linear regression.

RESULTS

Table 1: Comparison of insulin, glucose, TNF-α, and HOMA2 parameters

Parameters	Control (n=40)	FDRD (n=40)	Prediabetes (n=40)	Diabetes (n=40)	ANOVA P value
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
Fating Insulin (μIU/mL)	8.75±6.84	11.30±8.70	18.62±19.62	30.13±33.65	<.001
Fasting glucose (mg/dL)	86.75±9.25	89.35±6.53	114.05±6.81	158.75±15.84	<.001
TNF-α	9.78±7.11	15.02±11.93	19.50±19.70	37.15±39.57	<.001
HOMA2%B	108.72±66.72	119.87±60.25	101.35±65.46	83.56±76.36	.110
HOMA2%S	182.41±235.84	111.23±73.62	75.77±55.39	51.96±37.96	<.001
HOMA2IR	1.11±0.85	1.44±1.08	2.44±2.39	4.02±3.98	<.001

Fasting glucose, Fasting glucose, TNF alpha, HOMA2 %S and HOMA2IR were significantly different across the groups, while HOMA2%B was not significantly different across the groups (Table 1).

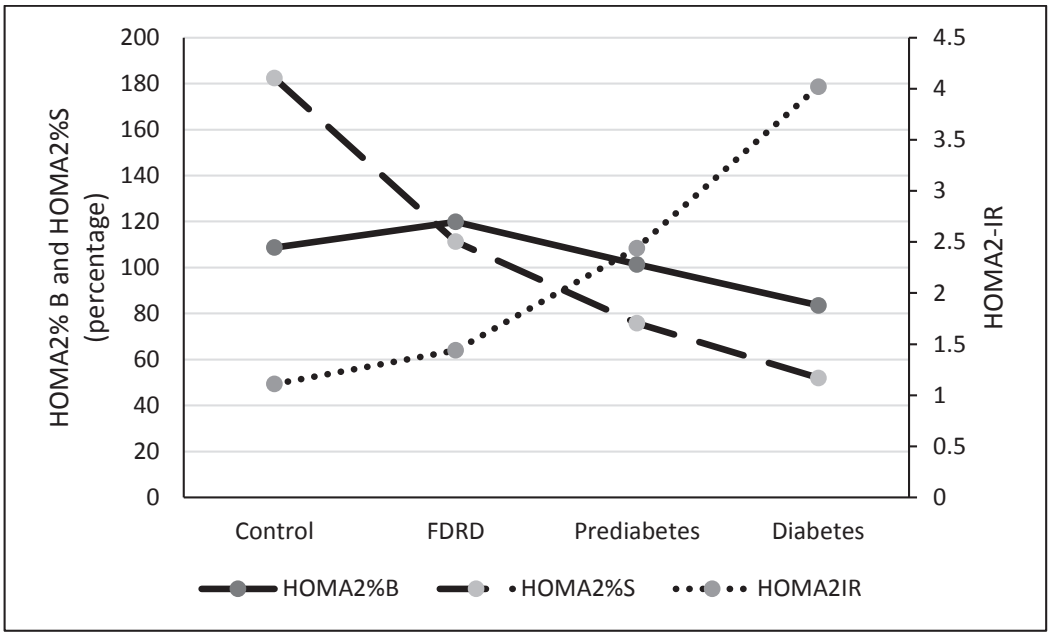

Figure 1: Relationship between insulin indices in the complete glycemic spectrum

From the values we can observe that Fasting insulin, fasting glucose, TNF-α, and HOMA2 IR gradually increases as we move from control, FDRD, prediabetes and diabetes, while HOMA2%S gradually decreases. HOMA2%B there is an increase in FDRD as compared to controls but decreases in prediabetes and diabetes (Figure 1 and Table 1).

Table 2: Comparison of Fasting insulin, fasting glucose, TNF-α, HOMA2 parameters – post-hoc analysis p values.

Parameters	Control vs FDRD	Control vs Prediabetes	Control vs Diabetes	FDRD vs Prediabetes	FDRD vs Diabetes	Prediabetes vs Diabetes
Fasting Insulin (μIU/mL)	.574	.031	<.001	.108	<.001	.012
Fasting glucose (mg/dL)	.261	<.001	<.001	<.001	<.001	<.001
TNF-α	.313	.062	<.001	.388	<.001	.001
HOMA2%B	.461	.626	<.001	.221	.017	.240
HOMA2%S	.014	<.001	<.001	.217	.407	.407
HOMA2IR	.545	.016	<.001	.068	<.001	.004

Post hoc analysis (Table 2): As compared to controls all the parameters (Fasting Insulin, Fasting glucose, TNF alpha, HOMA2%S, HOMA2 %B and HOMA2 IR) were significantly different in diabetes, except for HOMA2%B prediabetes group was also significantly different in all parameters, while FDRD was significantly different only in HOMA2%S while other parameters were comparable. As compared to FDRD diabetes were significantly different in all the parameters except for HOMA2%S, while prediabetes was significantly different only in glucose values. Prediabetes and diabetes groups were comparable based on HOMA2%B and HOMA2%S, while other parameters are significantly different.

Table 3: Pearson's correlation between insulin, glucose, derived insulin indices with TNF-α

Parameters	r value	TNF-α
Fasting Insulin (μIU/mL)	0.106	.181
Fasting glucose (mg/dL)	.414**	<.001**
HOMA2%B	-0.122	.126
HOMA2%S	-0.113	.156
HOMA2IR	0.129	.104

TNF-α shows significant positive correlation with fasting glucose (r = .414, p < .001, n =160) (Table 1 and Figure 1). There was no correlation between TNF-α, fasting insulin, HOMA2%B, HOMA2%S, and HOMA2IR (Table 3).

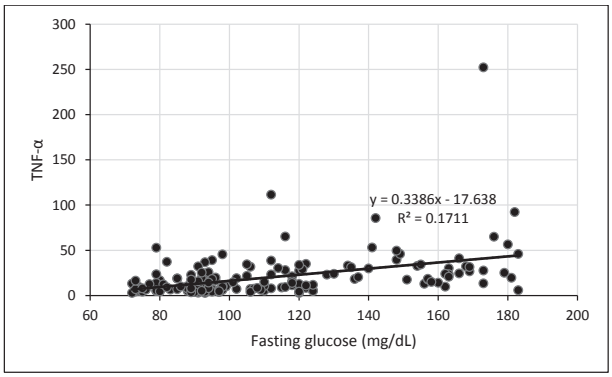

Figure 2: Correlation between TNF-α and fasting glucose

On regression analysis with TNF-α as dependent factor and fasting glucose as independent factor: TNF-**α** (pg/ml) = 0.3386 fasting glucose (mg/dL) -17.638. Only 17% of the changes in TNF-α could be explained by fasting glucose. After removing the seemingly outlier value of TNF-α (value - 252), we observed that correlation was more between TNF-α and Fasting glucose (r = .444, p < .001, n =159). However, on regression analysis only 19% (increase of 2%) of the changes in TNF-α could be explained by fasting glucose.

DISCUSSION

Diabetes is reported to be an immune mediated disease-causing cytokine mediated acute phase response and low-grade chronic inflammation leading to atherosclerosis and other complications [20]. TNF-α contributes in the development of insulin resistance, diabetes and altered adiposity [21]. Contradictory to this study one study have reported no association of inflammation in early insulin resistant state among non-obese first degree relatives of diabetes [22]. In view of these studies, it is essential to identify the association of TNF- α and insulin in complete glycemic spectrum.

Increasing TNF-α trend in the complete glycemic spectrum (Diabetes>Prediabetes> FDRD>Control group) suggests that low-grade subclinical inflammation starts even before the disturbance in glucose homeostasis (TNF-α: FDRD > control group), if there is a positive family history of diabetes. Inflammatory marker (TNF-α) have shown no correlation with insulin or insulin derived indices in our study. Whereas, we observed positive correlation with fasting plasma glucose. De Carvalho VF et al also have reported, association of hyperglycemia with inflammation, which agrees with our study findings [23]. The elevated levels of inflammatory marker and insulin prevails in diabetes regardless of their treatment (oral hypoglycemic agents). Despite the elevated levels of inflammatory marker and insulin there is no association between these two parameters with which we hypothesize that, severity of other pathophysiological mechanisms such as family history of diabetes, hyperglycemia, hyperinsulinemia [24, 25], body fat mass, glucose toxicity [24, 26] involved in insulin resistance could have masked the association of TNF- α and insulin resistance. Similar hypothesis is reported by another study which failed to show correlation between TNF- α and insulin resistant state in normoglycemic subjects [17]. Even in diabetic individuals, Darko et al have reported varying levels of TNF- α and IL-6 depending on demographic status (urban and rural) and hypothesized that it could be due to varying physical activity levels and body composition [27]. Even in our study only 17% of the variation in TNF-α could be explained by glucose levels.

Existing literature have documented insulin resistance in young lean subjects with family history of diabetes [28] which suggests the role of family history and no association between inflammation and insulin resistance among first-degree relative of diabetes [22]. This emphasizes the potential role of heritability leading to insulin resistant state rather than inflammation. These earlier suggestions support our study findings. Memon *et al*. have reported that among except for IL-6 no other cytokine (IL)-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12 (p70), IL-13, interferon-γ and TNF- α showed association with insulin sensitivity [29]. In a similar study, Herder et al have concluded that subclinical inflammation (IL-6,

hscrp) is associated with increased insulin resistance and fasting insulin levels even in non-diabetic individuals [30]. However, they have not measured TNF- α. The lack of association between TNF- α and insulin resistance/ fasting insulin might be due to the modest sample size in our study groups.

A study from Korea documented that concentration in serum TNF-α in prediabetic subjects were comparable with control group [12], which is in accordance with our study findings. This could be due to exclusion of morbid obese subjects in our study, because the major source of TNF-α is from adipocytes [31]. However, non-significant elevation of TNF- α and significant hyperglycemic state indicates that subjects with prediabetes have high risk for developing cardiovascular disease and diabetes respectively.

Hyperglycemic condition is associated with increased oxidative stress which in turn induces redox-sensitive major pro-inflammatory transcription factor nuclear factor kappa B (NFκB), leading to inflammation [32, 33]. From our study findings, we could say that, hyperglycemia have been implicated in the process of inflammation than insulin resistance across glycemic spectrum. Taken together, the relationship between hyperglycemia, oxidative stress and inflammation is analogous with bidirectional causation. Although, we could not find any significant association between insulin resistance and TNF-α. The increasing trend of insulin levels and TNF-α in FDRD, prediabetes and diabetes imply the influence of heritability and shows that the inflammatory cascade pathway and insulin resistance pathway occurs simultaneously with a missing link which remains unresolved.

Conclusion: Inflammation begins even in first degree relatives of diabetes and increases along with glucose levels along the glycemic spectrum.

Limitations: There are various confounding factors such as physical activity level, level of stress, occupation that could have influenced the level of inflammation in the study subjects which were not matched.

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Disclosure: We are presenting here only a part of a larger PhD project

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