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Original Research Article

The Influence of Inflammation on HBA1C Levels and Insulin Resistance in Prediabetes: Insights from Inflammatory Biomarkers

Ikshita Sabharwal[1], Amisha Bawa[2], Sukhraj Kaur[3]

[1,2]MBBS Student, Government Medical College Amritsar, Punjab, India
[3]Assistant Professor, Government Medical College Amritsar, Punjab, India

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Corresponding author: Ikshita Sabharwal
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Abstract:

This study aimed to investigate changes in inflammatory markers, including Interlekin-6 (IL-6) and Tumour Necrosis Factor-alpha (TNF-α), and triglyceride levels in individuals with prediabetes and their potential correlations. A total of 50 subjects, classified as prediabetic cases and controls, were selected based on their glycosylated haemoglobin (HbA1c) levels. The study found elevated levels of TNF-α and triglycerides in prediabetic individuals compared to healthy controls. Additionally, positive correlations were observed between HbA1c, TNF-α and IL-6, indicating the influence of inflammation on HbA1c levels and progression towards insulin resistance. Gender differences were also observed, with lower IL-6 levels in females and stronger positive correlations between IL-6 and HbA1c in males, and TNF-α and HbA1c levels in females. These findings highlight the importance of identifying biomarkers and early interventions in prediabetes to prevent diabetes and associated complications. Monitoring pro-inflammatory markers can aid in risk stratification, predicting complications, and tailoring therapy according to factors such as gender and age, leading to improved healthcare outcomes.

Keywords: prediabetes; tumour necrosis factor alpha; interleukin-6; glycosylated haemoglobin; triglycerides.

Introduction

Prediabetes is defined by blood glucose levels that are higher than average but not yet regarded as being in the diabetic range. [1] It is an intermediate stage between the normal glucose metabolism and the diagnosis of diabetes, also known as impaired glucose metabolism. Lifestyle modifications and early interventions play a huge role in checking the progression towards the diabetic state. According to a STEPS survey [2] conducted in 2017, the prevalence of prediabetes was found to be 6.3% (5.4 - 7.3%) in the North Indian state of Punjab based on American Diabetes Association (ADA) guidelines.[1] The laboratory measurements that can be used to detect prediabetes are fasting blood glucose (FBG), HbA1c, or 2-hour post load blood glucose (2hBG). [3]

The balance of inflammation is crucial for human health, as appropriate levels enhance immunity, while chronic inflammation is linked to various metabolic disorders such as type 2 diabetes, obesity, and cardiovascular disease, with higher levels of inflammatory markers correlating with disease progression and severity. [4] It is possible for inflammatory cytokines to worsen insulin resistance, prediabetes, and ultimately lead to type 2 diabetes mellitus. [5] Clinical findings have suggested that IL-6 and TNF-α play a role in glucose homeostasis and metabolism, and possibly influencing pancreatic β-cell function, leading to inflammatory responses, making them potential markers for predicting type 2 diabetes mellitus. [6]

The variation of inflammatory mediators like IL-6, TNF-α, and Triglyceride levels has been extensively investigated in individuals with Type 2 Diabetes Mellitus. Nevertheless, there is a paucity of research exploring the changes in these biomarkers and their interrelationships in individuals with Prediabetes. Hence, the present study aims to investigate the changes in IL-6, TNF-α, and Triglyceride levels in individuals with Prediabetes, as well as to examine their potential correlations.

Materials and Methods

The study was conducted at Guru Nanak Dev Hospital, Amritsar from April 29, 2023 to May 26, 2023 as part of our short term research elective. Subjects chosen were patients visiting the Medicine outpatient department who had undergone a screening for glycaemic control (FBG, HbA1c). Smokers, alcoholics, people with diagnosed diabetes; diabetic complications, chronic liver diseases, chronic renal diseases, Down's syndrome, acromegaly, and those on oral hypoglycaemic drugs were excluded from the study.

50 such subjects were chosen, of which 25 prediabetics with HbA1c levels between 5.7 and 6.4 % were classified as cases and the remaining 25 with HbA1c less than 5.7 % were classified as controls for this study, according to ADA criteria. [1] The patients were further segregated based on various factors as mentioned in the proceeding sections. Those found to be prediabetic were called again for further investigations (IL-6, TNF-α, and Triglycerides). HbA1c levels were detected by column chromatography, IL-6 and TNF-α were measured using enzyme-linked immunoassay (ELISA), and Triglycerides by a fully automated analyser.

Data thus collected were analysed statistically using the student's t test for significance of variance and Analysis of variance (ANOVA) to find correlation between the various parameters. Data are expressed as Mean ± SD. $p<0.05$ was considered statistically significant. Informed consent was obtained from all the subjects. The study was approved by the institutional ethics committee.

Results

Biochemical profile of patients and controls is presented in Table 1.

Table 1: Biochemical profile of patients and controls

S.NO	GROUP	HbA1c % Mean±SD	TNF-α pg/ml Mean±SD	IL-6 pg/mL Mean±SD	Triglycerides mg/dL Mean±SD
1	Patients	6.01±0.170*	51.08±63.98*	4.64±2.21*	167.92±51.58*
2	Controls	4.86±0.70	31.59±14.49	5.02±1.74	148.73±56.42
*p<0.05 when patients and controls were compared with each other.					

We categorised the patients into two groups according to their HbA1c levels. Group 1 comprised individuals with HbA1c levels ranging from 5.7% to 5.97%, while group 2 included individuals with HbA1c levels ranging from 6.0% to 6.30%. The study focused on three parameters, namely TNF-α, IL-6, and triglycerides, as illustrated in Table 2.

It was observed that mean HbA1c in group 1 was 5.85 ± 0.08 % and in group 2, it was 6.132 ± 0.10 %. There wasn't much variation in the levels of IL-6 between the two groups [4.62±1.87 pg/mL in group 1 vs. 4.19±2.22 pg/mL in group 2, p=0.66]. TNF-α showed a decreasing trend with HbA1c. Group 1 mean TNF-α levels were higher [55.35 pg/mL vs. 33.49 pg/mL] than group 2, although the difference was statistically insignificant [p=0.42]. Triglycerides did not vary significantly in the two groups [155.94 mg/dL in group 1 vs. 150.34 mg/dL in group 2, p=0.81]. A positive correlation was observed between HbA1c, TNF-α and triglycerides in group 1, and HbA1c, IL-6 and TNF-α in group 2. In group 1, IL-6 correlated negatively with HbA1c. Whereas in group 2, triglycerides showed a negative correlation with HbA1c.

Table 2: Comparison of various parameters according to HbA1c

S. NO	Group	HbA1c % Mean±SD	TNF-α pg/mL Mean±SD	IL-6 pg/mL Mean±SD	TRIGLYCERIDES mg/dL Mean±SD
1	5.7-5.97 %	5.85±0.08	55.35±71.63*	4.62±1.87**	155.94±44.33***
2	6.0-6.30 %	6.132±0.10	33.49±9.76#	4.19±2.22##	150.34±57.28###
*r =0.10, **r =-0.15, ***r = 0.31, #r = 0.12, ##r = 0.12, ###r = -0.15					

Further, we divided the individuals in group 2 into two subgroups depending on their gender. Mean IL-6 levels were significantly lower in females [1.64 pg/mL in females vs. 5.18 pg/mL in males, p=0.02] and correlated positively with HbA1c. TNF-α had a strong positive correlation with HbA1c in females. In males, TNF-α and triglycerides showed a negative correlation with HbA1c, whereas IL-6 had a positive correlation. The negative correlation between triglycerides and HbA1c was stronger in females.

Table 3: Comparison of various parameters according to Gender

S.NO	Group	HbA1c % Mean±SD	TNF-α pg/mL Mean±SD	IL-6 pg/mL Mean±SD	TRIGLYCERIDES mg/dL Mean±SD
1	Males	6.14±0.11	34.51±9.84*	5.18±1.53**	175.83±54.79***
2	Females	6.12±0.13	31.95±12.64#	1.64±1.46##	117.16±47.22###
*r = -0.31, **r = 0.49, ***r = -0.20, #r = 0.98, ##r = 0.15, ###r = -0.53					

Discussion

From a public health and clinical perspective, it is crucial to define and identify this pre-diabetic group since current research reveals that diabetes and cardiovascular disease prevention is most effective when applied early in the disease process. [3]

Assessment of chronic glycaemic levels, such as the HbA1c, reflects the extent of glucose exposure over time and is more closely connected to the risk of complications than discrete or incidental assessments of glucose levels. [7]

Increasing data indicate that obesity, insulin resistance, and related metabolic illnesses including Type 2 Diabetes may be influenced by persistent activation of pro-inflammatory pathways in the target cells of insulin action. [8] The identification and characterization of pertinent biomarkers and the elucidation of their functional significance in the underlying pathological mechanisms of the condition can facilitate the monitoring of disease advancement (or regression) and enable the prediction of clinical outcomes. This knowledge aids in the timely implementation of health measures. Thus far, there have been a limited number of investigations focusing on pro-inflammatory markers in individuals with pre-diabetes and there is a lack of consensus among various studies in the existing literature.

The present study evaluated the role of TNF-α, IL-6 and triglycerides in prediabetics and their correlation with HbA1c levels. In our study, patients exhibited elevated levels of TNF-α and triglycerides. Our findings support the previous studies which have consistently demonstrated that individuals with pre-diabetes have elevated levels of triglycerides and TNF-α when compared to healthy individuals. [5,9,10] These findings suggest that there are ongoing inflammatory processes in the body early in the course of the disease.

After categorising the prediabetic patients based on their increasing HbA1c levels, no significant differences were found in the levels of triglycerides, TNF-α and IL-6 among the 2 groups. Positive correlations were however found between HbA1c, TNF-α and IL-6, as HbA1c levels increased in group 2, thus indicating that inflammation, as depicted by IL-6 and TNF-α, is affecting the levels of HbA1c and that the individual is progressing towards insulin resistance. [4,6] Triglycerides initially correlated positively with HbA1c, which is supported by studies showing elevated triglycerides accompanying prediabetic states [9,11,12] but a negative correlation was observed at higher HbA1c levels. This might have been observed due to possible lifestyle interventions by the prediabetic subjects to reduce blood glucose and hence HbA1c levels.

Sexual dimorphism plays a significant role in modulating inflammatory processes and the subsequent biological response, and consequently, these sex differences can influence the impact of inflammation on metabolic processes in distinct ways between males and females. [13] In our study, further subdivision based on gender revealed significantly lower IL-6 levels among females with HbA1c $\geq$ 6 and the correlation between IL-6 and HbA1c was positive in both, but stronger among males. This is also confirmed by previous studies that demonstrated lower IL-6 levels in females. [13–15] This suggests that IL-6 as a biomarker for acquiring diabetes is implicated more strongly in males.

We also found that TNF-α correlated positively with HbA1c in females, and negatively in males. A previous study also demonstrated a suppressive effect of IL-6 on TNF-α, [14] thus supporting our observations that lower IL-6 levels in females are associated with higher TNF-α levels as mean HbA1c levels increase. Oestrogen plays a significant role in immunometabolism by preventing low-grade inflammation via direct inhibition of the secretion of cytokines derived from leukocytes. [13] Differences in fat deposition, influenced by estrogens, also contribute significantly to the observed divergence in inflammatory mediators between sexes. [13] Males exhibit higher triglyceride levels compared to females. [16]

These variations in triglyceride levels are attributed to the protective effect of estrogens.[17] In our study triglycerides were found to be higher in males, but the difference did not reach statistical significance. People with prediabetes typically have microvascular problems linked to hyperglycaemia, such as retinopathy, neuropathy, and nephropathy. [3] In a significant meta-analysis with a follow-up period of up to 24 years, the relative risk of diabetes in prediabetic cohorts was found to be 5.55. [3] People with elevated HbA1c had an increased risk of composite cardiovascular events, coronary heart disease and chronic kidney disease. [18,19]

Diabetic complications thus have long-term effects that lower quality of life and significantly increase healthcare costs because it is a chronic condition. [19] Clinical studies have shown that people with prediabetes can successfully prevent diabetes by making substantial changes to their lifestyle via dietary adjustments and increased physical activity. [3] Diabetes risk reduction has also been reported in various trials involving the use of pharmacotherapy as an early intervention. [3]

This calls for early diagnosis of prediabetes via the use of appropriate screening measures and prompt interventions which include lifestyle modifications and/or pharmacotherapy depending on the glycaemic profile of the patient, along with other contributory factors such as gender and age. Monitoring of pro-inflammatory markers can serve as a tool for risk stratification and predicting complications so as to reduce the all-cause mortality and morbidity associated with diabetes.

Conclusion

In conclusion, the present study highlights the importance of identifying and characterising biomarkers in individuals with prediabetes. Although the differences in inflammatory mediators between prediabetic patients and healthy individuals were not statistically significant, there is a consistent body of evidence indicating elevated triglyceride and TNF-α levels in prediabetes.

These findings suggest the presence of ongoing inflammatory processes early in the disease progression. Moreover, sex differences play a role in modulating inflammatory responses, with lower IL-6 levels observed in females. The protective effect of oestrogens is implicated in the divergence of inflammatory mediators between sexes. The study also emphasises the significance of early intervention and lifestyle modifications in preventing diabetes, as well as the importance of monitoring pro-inflammatory markers for risk stratification and predicting complications. Timely diagnosis and interventions targeting prediabetes can have a substantial impact on reducing the long-term burden of diabetes and improving healthcare outcomes.

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