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Original Research Article

Correlation between Various Inflammatory Markers, Serum Triglyceride Levels and Glycosylated Hemoglobin in Type II Diabetes Mellitus: A Case Control Study in an Indian Tertiary Care Setting

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Abstract:

The present study aims to assess the various biochemical parameters between 25 clinically diagnosed type 2 diabetics and 25 controls. We had divided the diabetics into 3 groups (on the basis of their serum glycosylated hemoglobin (HbA1c) levels) and also made a gender-based analysis of each group, to find the correlation between serum interleukin-6 (IL-6), tumor necrosis factor (TNF-alpha) and triglyceride levels (TG) with glycosylated hemoglobin (HbA1c). IL-6 showed a positive correlation with glycosylated hemoglobin in group 1,2 and a negative correlation in group 3. TNF-alpha showed a negative correlation in group 1 and a positive correlation with glycosylated hemoglobin in group 2 and 3. Serum triglycerides showed a negative correlation with glycosylated hemoglobin in all the three groups. The current study showed that diabetes is a state of inflammation and there exists an imbalance between the inflammatory markers and an overall higher serum triglyceride level in diabetics as compared to the normal controls. However, we stress on the need of further studies with regards to measurements of inflammatory markers to come across a specific coherent pattern between the rise/fall of the cytokines as the disease progresses, to consider anti-inflammatory drugs as potential therapies to decrease the progression of diabetes mellitus and the measurement of these markers to quantify risks in diabetic patients.

Keywords: Diabetes Mellitus; Interleukin-6; Tumor Necrosis Factor-Alpha; Glycosylated Hemoglobin; Triglycerides.

Introduction

Diabetes is a chronic, metabolic disease occurring either due to deficient insulin production/secretion or due to insulin resistance, which eventually leads to serious organ damage, the organs being- the heart, blood vessels, nerves, kidneys and eyes [1].

The diagnosis of diabetes, as per the American Diabetes Association (ADA) Guidelines [2] can be made either by an HbA1c test, measuring the average blood glucose levels over the past 2-3 months, where an HbA1c level of more than 6.5 g/dL and fasting plasma glucose (FPG) level of 126 mg/dL or more is indicative of diabetes. Other significant tests such as: an oral glucose tolerance test (2 hourly blood sugar levels of greater than or equal to 200 mg/dL) and a random blood sugar level of greater than or equal to 200 mg/dL are indicative as well [2].

As per the International Diabetes Federation (IDF) Atlas 10th ed. 2021 data, the global prevalence of diabetes is increasing. 1 in 10 adults suffer from diabetes (that being 537 million in total) globally, out of which 6.7 million die every year that is one death in every 5 seconds. The prevalence is estimated to rise to 643 million by 2030 and 783 million by 2045.

As per IDF Atlas's India Diabetes Report, the current prevalence (2021) is approximately 74 million (out of which around 65000 died in 2021) which is expected to rise up to 92.9 million by 2030 and 124.8 million by 2045. 53% of the people remain undiagnosed and the diabetes expenditure alone leads to an investment of approximately 85 million USD on healthcare in India. Such significant numbers render it necessary to dive deep into the pathophysiology and the factors associated with the progression of this disease [3]. A recent study has observed the prevalence of diabetes to be 14.3% in Punjab, the state where this study was conducted. Out of this, 34.2% were aware of their disease, 28.2% were receiving treatment and merely 14.2% were satisfactorily treated with controlled blood glucose levels[4].

Inflammation plays a vital role in the pathogenesis of type 2 diabetes mellitus (T2DM). Its role in the causation of diabetes, obesity and various cardiovascular metabolic diseases has been well established through multiple studies. As adipocytes are in close proximity to the immune cells, both low grade inflammation and meta inflammation (due to obesity caused by overeating) exist in T2DM [5].

Objective: Although the role of inflammatory markers of the chemokine (C-C motif) ligand (CCL) family, especially CCL2 (monocyte chemoattractant protein-1 (MCP-1)) in the progression of prediabetes to type 2 diabetes mellitus (T2DM) has been well established [6], but insufficient data exists with respect to IL-6, TNF-alpha and circulating serum triglyceride levels.

We hence aim to inspect the relation between IL-6, TNF-alpha and serum triglycerides in the causation of type 2 diabetes in adults.

Materials and Methods

This cross-sectional study was conducted from 29th April 2023 to 29th May 2023 at Guru Nanak Dev Hospital, Amritsar, Punjab, India with 25 patients who had a clinical and laboratory diagnosis of type 2 diabetes mellitus and 25 controls (n=50) with normal fasting blood sugar (99 mg/dL or lower) as a part of our short-term research elective project. All the patients matching our criteria and who gave informed consent were included in the study and the proposal was approved by the ethical committee of our college (in accordance with the principles of Declaration of Helsinki).

Inclusion Criteria: All the patients clinically diagnosed with type 2 diabetes mellitus and those who provided their consent to be a part of this study.

Exclusion Criteria: Patients who were diagnosed with type 1 diabetes mellitus (T1DM), on corticosteroid therapy, taking anti-diabetic drugs, lipid lowering drugs, nephrogenic diuretics, with a history of drug abuse, alcohol intake, smoking, recent infection suffering from acromegaly, Down's Syndrome, rheumatoid arthritis, pregnant females and cancer patients.

All the samples (patients and controls) were analyzed for serum glycosylated hemoglobin level using column chromatography, interleukin-6 (IL-6) using sandwich ELISA, tumor necrosis factor-alpha (TNF-alpha) levels using sandwich ELISA, serum triglyceride levels using fully automated analyzer. Data thus collected was analyzed statistically using the student t-test (significant of variance) and ANOVA to find correlation between different parameters. Values were expressed as mean ± S.D and p-value less than 0.05 was considered as statistically significant.

Results

Out of the 50 subjects chosen, we had 25 clinically diagnosed type 2 diabetes mellitus cases (HbA1c of 6.5% or higher as per the ADA guidelines) and 25 controls. The cases were further segregated on the basis of their serum HbA1c levels into 3 groups, the findings in each group being further analyzed gender-wise.

Various biochemical parameters assessed in the T2DM cases and controls have been mentioned in Table 1:

Table 1: Comparison of various parameters in 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	8.13 ± 2.05	34.30 ± 12.88*	3.54 ± 2.39**	202.58 166.89***
2	Controls	4.86±0.70	31.59±14.49#	5.02±1.74##	148.73±56.42###
*r=0.19, **r=-0.29, ***r= -0.14, #r=0.05, ##r=-0.42, ###r=0.01					

The cases had an overall higher HbA1c, higher TNF-alpha, higher serum triglycerides but lower IL-6 than the controls. All the people diagnosed with diabetes mellitus type 2 (T2DM) were divided into 3 groups on the basis of their serum HbA1c levels. Group 1: 6.5 to 7 g/dL, Group 2: >7 to 8 g/dL, Group 3: more than 8 g/dL

Table 2: Various parameters observed in the different groups

S.No.	Group [HbA1c levels in g/dL]	HbA1c [Mean ± SD]	TNF-α (pg/mL) [Mean ± SD]	IL-6 (pg/mL) [Mean ± SD]	Triglycerides (mg/dL) [Mean ± SD]
1	6.5-7	6.83 ± 0.13	29.18± 10.77*	5.10± 2.57**	196.27± 139.82***
2	7-8	7.63 ± 0.33	41.41± 14.56#	1.90± 1.76##	190.05± 101.60###
3	>8	10.42 ± 2.49	35.52± 12.70^	2.71± 0.99^^	222.34± 252.03^^^
*r=-0.13 **r=0.15,***r=-0.21, #r=0.65 ##r=0.36 ###r=-0.56, ^r=0.18 ^^r=-0.30 ^^^r=-0.36					

Among the three groups it was observed that the mean glycosylated hemoglobin levels rose, signifying progression to an uncontrolled state. The mean TNF-alpha values first increased then decreased as the disease worsened (The p value

being significant i.e., 0.5 in group 1, 2 and non-significant in group 2, 3 and group 3, 1).

The mean values of IL-6 initially dropped significantly followed by a mild increase as the disease worsened, the p-value being non-significant in all the groups. The serum triglycerides showed a minor variation in group 1 and 2, followed by some rise in group 3, with a significant p value in all the three groups (Group 1,2: 0.9; Group 2,3: 0.73; Group 3,1: 0.81).

It was observed that in **Group 1**, TNF-alpha and serum triglycerides had a negative correlation (-0.133 and -0.209 respectively) with HbA1c levels, whereas IL-6 had a positive correlation with HbA1c levels (0.146).

In **Group 2**, Both IL-6 and TNF-alpha had a positive correlation (0.356 and 0.654 respectively) with HbA1c whereas serum triglycerides had a negative correlation with glycosylated hemoglobin (-0.557).

In **Group 3**, it was observed that IL-6 and serum triglycerides showed a negative correlation (-0.304 and -0.360 respectively) with glycosylated hemoglobin whereas TNF-alpha had a positive correlation with HbA1c levels (0.183).

Table 3: Correlation between the parameters according to gender in group 1

S.No.	Group (6.5-7 g/dL)	HbA1c [Mean ± SD]	TNF-α (pg/mL) [Mean ± SD]	IL-6 (pg/mL) [Mean ± SD]	Triglycerides (mg/dL) [Mean ± SD]
A.	Males	6.85±0.09	26.05±8.97*	4.88±2.33**	209.03±155.39***
B.	Females	6.76±0.29	41.69±9.38#	5.98±4.42##	145.20±20.36###
*r=-0.34, **r=-0.41,***r=-0.37, #r=1, ##r=1, ###r=-1					

As mentioned in table 3 (i.e. Group 1 of table 2), females had higher mean levels of TNF-alpha than males. Although males showed a negative correlation (-0.34) and females showed a positive correlation (1) between TNF-alpha and glycosylated hemoglobin.

The difference between mean serum glycosylated hemoglobin levels was very little between both genders. Females showed a higher mean IL-6 levels than males, where males showed a negative correlation (-0.417) and females showed a positive correlation (1) between IL-6 and glycosylated hemoglobin (similar trend like the TNF-alpha).

With respect to mean serum triglycerides levels, females had lower levels than males. Both males and females showed a negative correlation (-0.375 and -1 respectively) between serum triglycerides and HbA1c levels.

Table 4: Correlation between the parameters according to gender in group 2

S.No.	Group (>7-8 g/dL)	HbA1c [Mean ± SD]	TNF-α (pg/mL) [Mean ± SD]	IL-6 (pg/mL) [Mean ± SD]	Triglycerides (mg/dL) [Mean ± SD]
A.	Males	7.45± 0.24	39.76± 18.49*	0.97± 0.84**	202.68± 110.90***
B.	Females	8± 0	44.72± 1.19#	3.27± 2.88##	164.8± 113.37###
*r= 0.94 **r=-0.83,***r=-0.86, #r= ##r= ###r=					

As mentioned in table 4 (i.e. **Group 2 of table 2**), both genders had a little variation in between mean HbA1c levels. Females had a higher mean TNF-alpha and IL-6 levels than males, but lower mean serum triglycerides levels.

Table 5: Correlation between the parameters according to gender in group 3

S.No.	Group (>8 g/dL)	HbA1c [Mean ± SD]	TNF-α (pg/mL) [Mean ± SD]	IL-6 (pg/mL) [Mean ± SD]	Triglycerides (mg/dL) [Mean ± SD]
A.	Males	8.96± 0.95	33.1± 11.60*	2.45± 0.32**	327.48± 398.49***
B.	Females	11.52± 2.84	37.34± 14.93#	2.91± 1.33##	143.49±42.70###
*r= 0.91, **r=-0.66, ***r=-0.74, #r=-0.03, ##r=-0.54, ###r=0.04					

As mentioned in table 5 (i.e. Group 3 of table 2), females had higher levels of mean glycosylated hemoglobin, TNF-alpha than males, where both the genders showed a positive correlation of TNF-alpha and HbA1c, but males had a higher positive correlation (0.91) than females (0.04). The variation between mean IL-6 levels between the both genders in group 3 was very little. With respect to mean serum triglyceride levels, females (mild positive correlation with HbA1c: 0.04) had a lower level than males (negative correlation with HbA1c: -0.748)

Discussion

On comparison of the three groups, that is group 1, 2 and 3, it was observed that both IL-6 and TNF-alpha did not follow a specific pattern of increasing or decreasing trend but IL-6 had a positive correlation with glycosylated hemoglobin in group 1,2: thus indicating that as the inflammation

increases, levels of glycosylated hemoglobin increase, whereas in poorly controlled diabetics who had a mean HbA1c of 10.42 ± 2.48 g/dL, a negative correlation with IL-6 was observed. This indicates that inflammation has already damaged the insulin secreting cells, hence raising the glycosylated hemoglobin. A study by Yin-Ling Chen showed that serum levels of IL-6 were higher than normal in patients diagnosed with T1DM (in subgroups of ethnicity, age and disease duration) 7, but here in T2DM, the mean IL-6 levels initially decreased as the disease progressed from mild to moderate stage, but then we observed a slight rise in mean IL-6 levels as the disease progressed to the severe stage (group 3). The mean IL-6 levels observed in patients were nevertheless less than the controls.

Another meta-analysis by Cheng concluded that a possible dose dependent correlation of -174 G/C mutant alleles, serum IL-6 levels with T2DM, supporting our findings of group 1, that is in the initial phase of diabetes, but the levels in the group 2 and 3 were in disagreement [8]. Moreover many studies have found higher levels of IL-6 in diabetic patients than the controls, contradictory to our findings [9, 10 ,11]. Another study also found no significant variation in between serum IL-6 levels in between diabetic cases suffering from nephropathies and controls [12]. A study by Anitha Balaji has compared the correlation of circulating IL-6 levels with T2DM and periodontitis and has shown that IL-6 levels tend to be the highest in uncontrolled T2DM and periodontitis, whereas in our study, as witnessed in group 3 (severely uncontrolled diabetes), the mean IL-6 levels were lower than those in the initial phase of diabetes [13]. Moreover, a study published in the year 2000, has although observed higher serum levels of IL-6 and TNF-alpha in diabetics (attributing this to the formation by adipose tissue cells) as compared to controls, but on culture the basal production of IL-6 was lower in diabetics than controls (attributing this to high triglyceride levels) [14]. They have also found a positive correlation between IL-6 levels and body mass index (BMI), but no other correlation between circulating IL-6 levels and any other variable was reached [14]. Furthermore a review by Louise Lang (2019) has mentioned that the IL-6 levels were higher at the onset of T2DM than in the patients suffering from chronic diabetes mellitus, backing up our findings in all the three groups with respect to the observed mean IL-6 levels and the correlation we found between IL-6 and HbA1c [15].

Inflammation plays an important role in the control of diabetes mellitus. With respect to TNF-alpha, a negative correlation was seen in group 1 but a positive correlation between glycosylated hemoglobin and TNF-alpha was observed in group 2 and group 3, as the disease worsens. It clearly depicts that inflammation is affecting glycosylation of hemoglobin, thereby leading to increased levels of glycosylated hemoglobin and increase in levels of TNF-alpha.

Serum triglycerides had a negative correlation in all the three groups thus indicating that as diabetes proceeds from mild control to poor control phase, the mobilization of fatty acids from the adipose tissue is increased to meet the energy demands of the cell. Many studies have shown that the levels of plasma triglycerides were higher than normal in diabetic patients as compared to healthy controls [16, 17], something which was observed here too in all the groups, where the mean serum triglyceride levels were higher in the patients (202.58 ± 166.89 mg/dL) than the controls (148.73 ± 56.42 mg/dL). One study has also shown that higher serum levels of free fatty acids prevail in patients suffering from T2DM. These higher levels than induce the release of TNF-alpha from macrophages, which support our findings [18].

However, another cross-sectional study has concluded no significant variation in genotype and allele frequency between diabetics and controls, advocating for the need of further investigations into the matter [9]. IL-6 brings about adiponectin suppression, induction of CRP (C-reactive protein) secretion (which brings about changes in the signaling pathways) which eventually leads to insulin resistance in diabetes [19]. Its positive correlation with glycosylated hemoglobin in mild and moderate diabetes signify that inflammation increases beta cell destruction and hence increased glucose levels and increased glycosylation of hemoglobin, but a negative correlation in the severe uncontrolled phase signifies, that inflammation has already caused major damage that with further glycosylation of hemoglobin, IL-6 will decrease.

The role of IL-6 in the progression of type 2 diabetes mellitus has always been a point of debate, and we too consider that further research is needed in this area. TNF-alpha promotes inflammation, suppresses adiponectin expression, promotes the expression of resistin, increases the release of free fatty acids, thereby promoting insulin resistance [20]. Moreover, TNF-alpha may stimulate the release of MCP-1, whose role in the causation of insulin resistance has been well established by past studies [21]. A positive correlation of TNF-alpha with glycosylated hemoglobin as the disease worsens (in group 2,3), suggests that TNF-alpha mediated inflammation plays a more crucial role than IL-6 in the later stages of diabetes mellitus.

Obesity bears a predisposition to type 2 Diabetes Mellitus, since adipose tissue secretes many pro-inflammatory cytokines like TNF-alpha, IL-6, IL-1, retinol-binding protein (RBP), resistin, amyloid

etc., which eventually brings about insulin resistance [20]. Obesity further creates a hypoxic state, which sets off inflammation [20]. Although the serum triglycerides levels were higher than normal in the control, they showed a negative correlation with glycosylated hemoglobin, suggesting higher fat mobilization to fit in with the increased energy requirements of the body. Moreover, insulin promotes glucose transport across the cells. In case of type 2 diabetes due to low insulin levels (where inflammation has destroyed the pancreatic beta cells), glucose transport across the cells is hampered, further justifying the increased demand of triglycerides in meeting the energy demands of the cells.

Hence, appropriate dietary interventions to control the circulating triglyceride (hence the adiposity) and blood glucose levels are deemed necessary in order to control inflammation and the progression of diabetes mellitus to an uncontrolled state.

Conclusion

Taking into consideration the lifelong complications, a rapid rise in incidence and prevalence, increasing morbidity and mortality and the rising health expenditure due to diabetes, it's rendered necessary to dig deep into the underlying pathophysiology, especially the role of inflammation and various biochemical parameters in its progression.

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Limitations of the Study: Our sample size, due to time constraints, was small. The subjects were chosen from the outpatient department of Guru Nanak Dev Hospital, Amritsar, Punjab, India which is a tertiary health care center catering to the health needs of a mixed variety of population from both urban and rural backgrounds (usually with advanced disease), where the people from rural backgrounds are not so disclosive about other health conditions that they may be suffering from.

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