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Inflammatory And Autoimmune Biomarkers With Chronic Sitagliptin Plus Metformin Users In Type 2 Diabetes Mellitus

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Abstract*: Background: Type 2 diabetes mellitus (T2DM) is characterised by chronic low-grade inflammation and immune dysregulation. Immunomodulatory effects of dipeptidyl peptidase-4 (DPP-4) inhibitors, such as sitagliptin, have been demonstrated, but the long-term relationship of these effects with inflammatory and autoimmune biomarkers is not well understood. In the current study, we studied the serum high-sensitivity C-reactive protein (hs-CRP) and antinuclear antibodies (ANA) in the patients with T2DM under chronic therapy with sitagliptin plus metformin. Methods: This prospective case-control study involved 85 subjects, including healthy controls (n = 30), patients with T2DM on chronic sitagliptin and metformin therapy (n = 25), and patients with T2DM on other antidiabetic medications (n = 30). Serum hs-CRP and ANA levels were determined using enzyme-linked immunosorbent assay (ELISA). Data analysis was performed using the Kruskal–Wallis test followed by the Mann–Whitney U test for pairwise comparison. Cohen's f was used to estimate effect sizes, and statistical significance was $P < 0.05$. Results: There were significant differences between the study groups in the serum levels of hs-CRP and ANA ($P = 0.0001$ and $P = 0.0219$, respectively). The highest hs-CRP concentrations were observed in patients on chronic sitagliptin plus metformin therapy, followed by patients on other antidiabetic medications, and the lowest levels were seen in healthy controls. Similarly, ANA levels were significantly higher in the sitagliptin plus metformin group than in either of the two comparison groups. The effect size observed was large for hs-CRP (Cohen's $f = 0.7$) and moderate for the ANA (Cohen's $f = 0.3$). Conclusions: Long-term treatment with sitagliptin plus metformin was associated with higher circulating levels of hs-CRP and ANA than in healthy controls and patients receiving other antidiabetic therapies. These results are in favour of ongoing inflammation and immune system changes in patients with T2DM and stress the need for larger prospective studies to elucidate the mechanisms underlying these associations and their possible clinical implications.*

Keywords—Type 2 diabetes mellitus; Sitagliptin; Metformin; High-sensitivity C-reactive protein; Antinuclear antibodies; Inflammation; Autoimmunity.

Introduction

Type 2 diabetes mellitus (T2DM) is a chronic metabolic syndrome with increasing prevalence in the world. Many lifestyle factors contribute to the development of T2DM, including physical inactivity, a sedentary lifestyle, cigarette smoking, and excess alcohol consumption. People with T2DMare more susceptible to different types of short- and long-term complications and often die prematurely [1]

Type 2 diabetes mellitus (T2DM) is commonly associated with increasing insulin resistance that results from obesity and chronic low-grade inflammation. Persistent inflammatory activity is often accompanied by the development of diabetic complications; Therefore, inflammatory biomarkers have been widely investigated in the evaluation of pathological changes associated with T2DM [1] .

High-sensitivity C-reactive protein (hs-CRP) is synthesized in the liver, under the regulation of proinflammatory cytokines (IL-6) and TNF-alpha. Increased hs-CRP is produced from hyperglycemia, obesity, and diabetic complications and can thus be used as a marker for inflammation and disease progression in type 2 diabetes mellitus [2].

Antinuclear antibodies (ANAs) are autoantibodies directed against various nuclear constituents and nucleoprotein complexes. ANAs are known to be important serologic markers of autoimmune diseases. ANA levels are increased due to immune dysregulation. Such oxidative stress and T2DM can induce structural changes in nuclear proteins and DNA, which may lead to neoantigen formation. Autoantibodies, such as ANA, can be triggered by neoantigens. Such mechanisms explain the link between chronic metabolic dysregulations of T2DM and autoimmune responses with high ANA levels [3, 4].

Sitagliptin is an oral antidiabetic drug used in the treatment of type II diabetes mellitus (T2DM). It is a member of the drug class of dipeptidyl peptidase-4 (DPP-4) inhibitors. New research suggests sitagliptin may have effects in reducing inflammation and modulating immune system activity. Sitagliptin has been shown to reduce the production of inflammatory mediators in the body and improve metabolic conditions associated with chronic inflammation [5]. DPP-4

(also known as CD26) is expressed on many immune cells. The activity of CD26 may alter immune function and play a role in autoimmune processes[6]. There is increasing evidence about the anti-inflammatory and immunomodulatory effects of sitagliptin. But the effect of sitagliptin on inflammatory and autoimmune biomarkers in T2DM patients is not well known. Very few studies have evaluated hs-CRP and ANA in patients taking long-term sitagliptin and metformin. Currently our study is required to determine the association of chronic sitagliptin administration with inflammatory and autoimmune responses in T2DM patients.

the present study aimed to evaluate the inflammatory biomarker hs-CRP and the autoimmune biomarker ANA in patients with T2DM receiving chronic sitagliptin plus metformin therapy and compare them with patients receiving other antidiabetic medications **and healthy controls.**

Materials and Method

Study design: The study population included 85 subjects in three groups: healthy controls (n = 30), patients with type 2 diabetes mellitus on chronic sitagliptin + metformin therapy (n = 25) and patients with type 2 diabetes mellitus on other antidiabetic drugs (n = 30). The patients were from Diabetes and Endocrinology Center, Thi-Qar Health Department, Iraq, 2025–2026. This was a prospective case control study. Inflammatory and autoimmune markers were tested for in the study groups .

Inclusion criteria

participants aged between 40–70 years. None of the participants had a history of malignancy or chemotherapy. Nonpregnant participants and with no autoimmune or thyroid diseases and without insulin therapy.

Exclusion Criteria

Patients with severe renal and hepatic impairment, history of alcohol abuse, or exposure to known carcinogenic agents were excluded from the study.

Table1 / materials, instruments and kits used in study

Materials/ instruments	The company manufacture	Country
Antinuclear antibodies (ANAs)	.ELK Biotechnology Co., Ltd.	China
High-sensitivity C-reactive protein (hs-CRP)	ELK Biotechnology Co., Ltd.	China
ELISA Reader	Biotek	USA
Centrifuge	Hettich	Germany

Sample Collection: Venous blood was collected from all the participants by aseptic techniques. After centrifugation, serum was aspirated and stored at -20°C until further analysis. Serum levels of high sensitivity C-reactive protein (hs-CRP) and antinuclear antibodies (ANA) were determined by ELISA kits using ELISA reader as per the instructions of the kit company.

Ethical Approval: The study was approved by the research committee of Training and Human Development Center, Thi-Qar Health Directorate, Ministry of Health, Iraq. The study was approved by the Human Research Ethics Committee of the Diabetes and Endocrinology Center on 07 October 2025 (Approval No. 35556). All subjects were informed about the aim of the study and written informed consent was obtained prior to enrollment. This study was conducted in accordance with the ethical principles of the Declaration of Helsinki.

Statistical analysis: Statistical analysis was done using SPSS software. The normality of the data was checked using the Shapiro–Wilk test. Results are expressed as median (range) since most of the variables were not normally distributed. Differences between study groups were analyzed using Kruskal-Walli's test and Mann-Whitney U test for pairwise comparisons. (Cohen's f) to estimate the difference between study groups. statistically significant if the p-value is < 0.05 .

Results:

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Table 1 / Charateristic of study population

parameter	Control n=30	Sitagliptin &metformin n=25	Other drugs n=30	P- valu
AGE				
Age	43 (48-40)	51(71-40)	54(72-42)	0,0001
Gender				
Male (%)	26 (86.7%)	11(44%)	19(63.3%)	0.0037
Female (%)	4 (13.3%)	14 (56%)	11(36.7%)	
Anthropometric measurements				
BMI	27.2(21.1-30.2)	28.5 (25.9-43.46)	28 (20.3-40)	0.0109
Glycemic control				
HbA1c %	5.4 (5-5.8)	7.2 (5.9- 11.4)	8.5 (6.1-10.6)	0.0001

Data that reject normal distributed expressed as Median (Range) tested by Independent-Samples Kruskal-Wallis Test

P value <0.05 considered significant

The Shapiro–Wilk test was used to check normality of the data. The results showed that the study variables were not normally distributed ($P < 0.05$). Therefore, data are expressed as median (range) and the difference between study groups was analyzed using the Kruskal-Walli's test. A P value of <0.05 was considered statistically significant.

Table 1 / Biomarkers of inflammintory and auto immune among study groups

Parameter	Control n=30	Sitagliptin & metformin n=2	Other drugs n= 30	Cohen's f	P-value
HS _CRP	44.5 (20.6-88.6)	95.4 (38.9-119.9) a	88 (61.9- 113) a	0.7	0.0001
ANA	3.1 (0.63 - 8.95)	6.98 (0.63-14.2) a	4.24 (0.24-10.2) b	0.3	0.0219

Data are presented as Median (Range) and tested by Independent-Samples Kruskal-Wallis Test P .value <0.05 considered significant

.a significant at P<0.05 as compared to Control values

b significant at P<0.05 when compared to Sitagliptin + Metformin group

Data of each pair of groups analyzed by Mann Whitney test

f = 0.1 small, 0.25 medium, =0.4 large effect

Table 3 shows the inflammatory and autoimmune biomarkers of the study groups. The difference between groups was statistically significant in the serum hs-CRP levels ($P = 0.0001$). The highest levels of hs-CRP were found in patients who were chronically treated with sitagliptin and metformin [95.4 (38.9–119.9)], followed by patients treated with other antidiabetic medication [88 (61.9–113)] and lowest in healthy controls [44.5 (20.6–88.6)]. The effect size of the difference was large (Cohen's $f = 0.7$). There was also a significant difference in ANA levels among the study groups ($P=0.0219$). Patients receiving chronic sitagliptin and metformin therapy had higher ANA levels [6.98 (0.63–14.2)] compared to both healthy controls [3.1 (0.63–8.95)] and patients receiving other antidiabetic medications [4.24 (0.24–10.2)]. The pairwise comparisons showed significant differences between the sitagliptin plus metformin group and the other study groups. ANA had a medium effect size (Cohen's $f = 0.3$) .

Discussion:

The present study measured inflammatory and autoimmune biomarkers in patients with T2DM treated long-term with sitagliptin plus metformin, patients on other antidiabetic drugs, and healthy controls. The results showed a significant difference between study groups as regards hs-CRP and ANA levels. In the study, patients on chronic sitagliptin plus metformin therapy had the highest hs-CRP and ANA levels among the other groups. These findings suggest that long-term treatment with sitagliptin plus metformin may be associated with changes in inflammatory and autoimmune activity in patients with T2DM. Further studies are needed to clarify the mechanisms and to determine the clinical importance of these results.

Elevated levels of hs-CRP may be caused by the chronic inflammatory status associated with T2DM.Chronic hyperglycemia and insulin resistance induce the secretion of proinflammatory cytokines, particularly IL-6 and activation of inflammatory pathways. IL-6 induces hepatic CRP synthesis[2]. The current study found that serum levels of hs-CRP were significantly elevated in T2DM patients compared with healthy controls. This observation is in concordance with Pradhan et al. and Wang et al they reported CRP as an independent predictor of T2DM and proposed that chronic inflammation has a crucial part to play in disease development[7, 8] .

Sitagliptin has been reported to have anti-inflammatory properties, but in the present study hs-CRP was highest in patients on chronic sitagliptin + metformin therapy. This may be reflective of the complex immunomodulatory activity of DPP-4 inhibition as opposed to a direct pro-inflammatory effect of sitagliptin. DPP-4 (CD26) is expressed on many immune cells such as T lymphocytes, B lymphocytes, macrophages and dendritic cells where it is involved in immune regulation. The mechanisms regulating immune responses through DPP-4 inhibition are still not well elucidated (Drakul and Colic, 2023). Shao et al obtained similar results, hypothesizing that the immunomodulatory effects of DPP-4 inhibitors may be associated with the inflammatory microenvironment and disease characteristics. Thus, the high level of hs-CRP in the sitagliptin-treated group may be a marker of ongoing low-grade inflammation associated with T2DM and a complex

immune regulatory effect of chronic DPP-4 inhibition rather than a direct drug effect[6, 9, 10] .

The current study revealed significantly increased ANA levels among patients on chronic sitagliptin plus metformin therapy as compared to healthy controls and patients on other antidiabetic medications. Increased ANA levels in T2DM may be due to persistent metabolic disturbances with oxidative stress and immune dysregulation. Chronic hyperglycemia induces an excessive generation of reactive oxygen species (ROS) resulting in oxidative modifications of nuclear proteins and DNA. Such structural changes may result in the formation of neoantigens that break immune tolerance and induce autoimmune responses resulting in production of autoantibodies including ANA. In addition, chronic inflammation and defective clearance of apoptotic cellular debris may prolong exposure of nuclear antigens to the immune system and thus facilitate autoantibody formation [11] . In a similar way, Nosal et al. and Krzemień et al. showed a strong correlation of antinuclear autoantibodies with markers of oxidative stress and explained role of oxidative injury in the development of autoantibodies. T2DM is accompanied with chronic inflammation and immune dysregulation. T2DM is linked to autoimmune responses and loss of self-tolerance. These mechanisms may explain the increased ANA levels in T2DM patients. [3, 4] .

Chronic therapy with sitagliptin + metformin was associated with the highest ANA levels. The exact mechanism is unknown, but this finding may be related to the immunomodulatory effect of DPP-4 inhibition and not a direct autoimmune effect of sitagliptin. DPP-4 (CD26) is expressed by a broad range of immune cells and is important for immune signaling and homeostasis. Huang et al. demonstrated that DPP-4 is a critical regulator of adaptive immunity and autoimmune pathways and that alterations in DPP-4 activity could influence immune tolerance and autoantibody production [10] .

Emerging evidence suggests that chronic metabolic inflammation may contribute to loss of self-tolerance and generation of autoantibodies. Valentino et al. have suggested that the chronic low-grade inflammation associated with obesity, aging, and metabolic diseases leads to immune dysregulation and the development of autoreactive immune responses[12]. Frasca et al . show that T2DM patients have B-cell dysfunction and systemic inflammation low antibody production and dysregulation of humoral immunity may produce from dysfunctional state. B-lymphocytes are the main producers of autoantibodies and altered B-cell function may be responsible for the increased ANA levels observed in this study. They have been associated with various immune mediated diseases. This implies that chronic inhibition of DPP-4 could affect immune pathways in susceptible individuals... [13]. Roy et al. reviewed the accumulating evidence of the association of DPP-4 inhibitors with autoimmune phenomena and suggested that modulation of DPP-4 activity may be associated with altered immune regulation rather than direct induction of autoimmunity[14]. The elevated ANA levels in the sitagliptin-treated group may be explained by chronic metabolic inflammation, B-cell dysfunction, and immune modulation with long-term DPP-4 inhibition.

The findings of the present study together suggest that patients with T2DM on long-term sitagliptin + metformin therapy display changes in inflammatory and autoimmune biomarkers with significantly increased levels of hs-CRP and ANA. These observations support the concept of chronic inflammation and immune dysregulation as important components of T2DM and may be influenced by long-term modulation of the DPP-4/CD26 pathway. While the precise mechanisms involved in these changes have yet to be fully clarified, the current results point to a potential interaction between metabolic disturbances, inflammatory response and immune regulation in patients with T2DM. Additional large prospective studies are needed to clarify the underlying mechanisms and to determine the clinical significance of this observation.

Study limitations:

There are several limitations of the present study that should be considered when interpreting the findings.

1-the relatively small number of participants included in this study may limit the possibility of generalizing the findings to all patients with T2DM

. 2-the significant differences between the study groups in age and gender distribution may be potential confounding factors.

3- this study was a case-control design and therefore cannot definitively establish a causal relationship between chronic sitagliptin use and alterations in inflammatory or autoimmune biomarkers. Moreover, inflammatory cytokines including IL-6 and TNF-α were not measured, which restricts further investigation of the underlying mechanisms that may account for the changes of hs-CRP and ANA levels seen in the current study.

4-ANA positivity was not further characterized by specific autoantibody profiles and therefore, the clinical significance of the elevated ANA levels is uncertain. These findings need to be confirmed and their clinical implications clarified in further large prospective studies.

Conclusions:

The present study showed significantly higher levels of hs-CRP and ANA in T2DM patients compared to healthy controls. The patients on long-term therapy with sitagliptin + metformin had the highest levels of both biomarkers in the study population. The results indicate that even with antidiabetic therapy, patients with T2DM might experience

ongoing chronic inflammation and immune dysregulation. The observed hs-CRP and ANA increases may be due to the complex interaction of metabolic disturbances, inflammatory responses and immune regulatory mechanisms. Further studies with larger sample sizes are needed to better understand underlying mechanisms and to establish the clinical relevance of these findings.

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