Original Article

Glycemic Control in Type 2 Diabetes Mellitus Patients Could Affect NLRP3 Inflammasome Level

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Abstract

Background: Inflammasome complex such as nucleotide oligomerization domain-like receptor family protein 3 (NLRP3) acts as a trigger initiating inflammatory responses and could lead to endothelial dysfunction in diabetic patients and glycemic control could affect the mitochondrial stress through NLRP3 inflammasome level. **Objectives:** This study was conducted to ensure that type 2 diabetes mellitus (T2DM) glycemic control could affect mitochondrial stress through NLRP3 inflammasome level leading to an aberrant immune response. **Materials and Methods:** A case–control study was conducted on 90 Iraqi subjects, 60 of them were T2DM who were subgrouped into 30 patients with good glycemic control (HbA1c ≤ 7%) and the second group with 30 patients with poor (bad) glycemic control (HbA1c > 7%). Also, 30 healthy control subjects were enrolled in this study. HbA1c, fasting blood glucose (FBG), and serum levels of NLRP3 and interferon (IFN)-γ were quantitatively determined by means of a sandwich enzyme-linked immunosorbent assay (ELISA) test. **Results:** Results of this study showed a significant increase in serum levels of NLRP3 and IFN-γ in the poor glycemic control group of patients as compared to control subjects ($P \leq 0.05$), while this difference was not significant when comparing the good glycemic control group of patients with control subjects. There is a significant positive correlation of serum NLRP3 with only IFN-γ ($P \leq 0.05$) in both good and poor glycemic control and healthy controls. **Conclusion:** An increased level of NLRP3 was observed in poor glycemic control T2DM and correlated with IFN-γ, suggesting hyperglycemia's effect on this inflammasome pathway that could be associated with aberrant cytokine induction, a key inducer of diabetic complications.

Keywords: Glycemic control, IFN-γ, inflammasome, NLRP3, type 2 diabetes mellitus

Introduction

Chronic low-grade inflammation is an associated pathology of type 2 diabetes mellitus (T2DM) with other factors such as insulin resistance obesity and cardiovascular diseases (CVD) and this chronic inflammation support idea of the immune system is a key player in the pathogenesis of T2DM.[1] The aberrant activation of the immune system especially the innate immune system in T2DM patients leads to chronic inflammation through the activation of the myeloid innate immune cells such as macrophages and their receptors and mediators, which play a role in identifying the danger signals of metabolites of T2DM signaling the production of pro-inflammatory cascade.[2] interleukin-1 (IL-1) family is known to be important systemic and vascular effectors that contribute to metabolic diseases such as atherosclerosis and T2DM,[3] suggesting that pro-inflammatory cytokines such as interferon (IFN)-γ, oxidative stress, and innate immune receptors all promote the progression of metabolic events by activating the inflammasome proteins such as nucleotide oligomerization domain-like receptor family protein 3 (NLRP3).[4] This complex consists of different types of multiprotein, which can be induced through the activation of nuclear factors of inflammation induced by pathogen-associated molecular patterns (PAMPs), and damage-associated molecular patterns (DAMPs).[4,5] The

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association of NLRP3 inflammasome activation with T2DM is of interest to be studied by a researcher as the key role of inflammasome activation in leukocytes of T2DM may differ from one patient to another that can be reflected by cytokine profile depending on glycemic status.[6]

Studies showed that NLRP3 plays an important role in metabolic diseases such as CVD, atherosclerosis, and T2DM[7] through the activation of these inflammasomes by abnormal metabolic stimuli of hyperglycemia induced by insulin resistance leading to the production of pro-inflammatory cytokines[8]

This study was conducted to ensure that T2DM glycemic control could affect the mitochondrial stress through NLRP3 inflammasome level leading to an aberrant immune response in this group of patients.

Materials and Methods

This case–control study was conducted on 90 Iraqi subjects, 60 of whom had T2DM and were attending the National Diabetes Center of Mustansiriyah University in 2022. The T2DM group was further divided into two subgroups: the first group consisted of 30 patients with good glycemic control (HbA1c ≤ 7%), while the second group consisted of 30 patients with poor (or bad) glycemic control (HbA1c > 7%).[9] People with frequent severe hypoglycemia, advanced complications, or low life expectancy were excluded from this study. The diagnosis was conducted by the consultant physicians in the center. Additionally, another 30 nondiabetic apparently healthy subjects were included in the study for comparative purposes. Blood samples were obtained from each subject after 10–12 h of fasting, for HbA1c and fasting blood glucose (FBG). The body mass index (BMI) was calculated by dividing weight (kilogram) by the squared height and the serum level of NLRP3 and IFN-γ was quantitatively determined by means of a sandwich enzyme-linked immunosorbent assay (ELISA) test using commercially available kits (SUNLONG-Biotech, Hangzhou, China) according to the manufacturer's instruction. In a sandwich ELISA, the test involves capturing the target analyte (NLRP3 or IFN-γ) between two specific antibodies—an immobilized capture antibody and a labeled detection antibody. The labeled detection antibody is usually linked to an enzyme that produces a color change when acted upon by a substrate. A standard curve is typically generated and constructed by measuring the absorbance values of a series of known analyte concentrations (standards) and plotting them against the corresponding concentrations. This generates a linear relationship between the absorbance values and the analyte concentrations. Then absorbance value of an unknown sample is measured. By comparing the absorbance value of the sample to the standard curve, the corresponding analyte concentration can be determined.

Statistical analysis

The Prism GraphPad software version 9.5.1 has been used for data analysis using a *t* test to obtain the differences between the means of two groups, whereas the analysis of variance (ANOVA) test was used to assess the differences between more than two groups. A *P* value of less than 0.05 is considered statistically significant. The Chi-square test (χ^2 test) was used to assess the difference between the percentage and considered significant at 0.05 level. The correlation coefficients between the different studied group parameters using Pearson analysis.

Ethical approval

The ethical approval of this study was observed according to the principle of the Declaration of Helsinki and after taking patients' verbal and analytical approval before the taking of samples. The study protocol, subject information, and consent form were reviewed and approved by a local ethics committee of the microbiology department in the medical college of Mustansiriytah University according to document number 98 on November 16, 2022 to get this approval.

Results

The patients were grouped into two subgroups: good glycemic control (HbA1C ≤ 7%) and bad glycemic control (HbA1c > 7%) statistical differences between means of studied parameters were assessed independently for T2DM patients and control subjects.

The mean age of diabetic patients with good glycemic control was 57.6 ± 1.85 (mean ± SE) ranging from 42 to 74 years and for bad glycemic control was 58.74 ± 1.42 (mean ± SE) ranging from 40 to75 years, while that of controls was 53.83 ± 1.6 ranging from 38 to 72 years. A comparable proportion of males (52.2% vs. 53.3%) and females (47.8% vs. 46.7%) were presented from T2DM patients and controls, respectively ($P > 0.05$).

The mean values of HbA1c and BMI in T2DM and control subjects showed a variation in the three groups, and this variation was significant ($P \leq 0.0001$ and 0.004, respectively). Serum level of NLRP3 and IFN-γ was significantly different in the three studied groups as shown in Table 1.

ANOVA-test significant difference between more than two independent means at the 0.05 level.

To investigate the impact of the glycemic state as defined by HbA1c, on the investigated parameters (BMI, NLRP3, and IFN-γ), statistical differences between means of these parameters were assessed. Such differences were assessed independently for T2DM patients and control subjects.

The graph in Figure 1A displays BMI levels and there was an obvious significant increase in diabetic patients with

Table 1: Mean values of investigated parameters in type 2 diabetes mellitus patients (good glycemic control and bad glycemic control) and controls

Variables	Control	Good Glycemic control	Poor (bad) Glycemic control	*P* value
Number of values	30	30	30	
HbA1c, % Mean ± SD (range)	4.84 ± 0.37 (4–5.6)	5.70 ± 0.71 (4.1–6.6)	8.92 ± 1.42 (7.2–12)	0.0001
BMI, kg/m^2 Mean ± SD (range)	27.25 ± 3.814 (19–34)	30.20 ± 4.183 (23–38)	31.71 ± 5.448 (21–43)	0.004
NLRP3, pg/mL Mean ± SD (range)	113.5 ± 5.399 (103–123.5)	116.2 ± 4.377 (105.5–123.9)	118.8 ± 8.881 (105.3–148)	0.04
IFN-γ, pg/mL Mean ± SD (range)	5.24 ± 1.995 (1.59–9.7)	6.32 ± 2.978 (2.3–15.4)	6.89 ± 2.424 (2.7–12.3)	0.05

SD: standard deviation

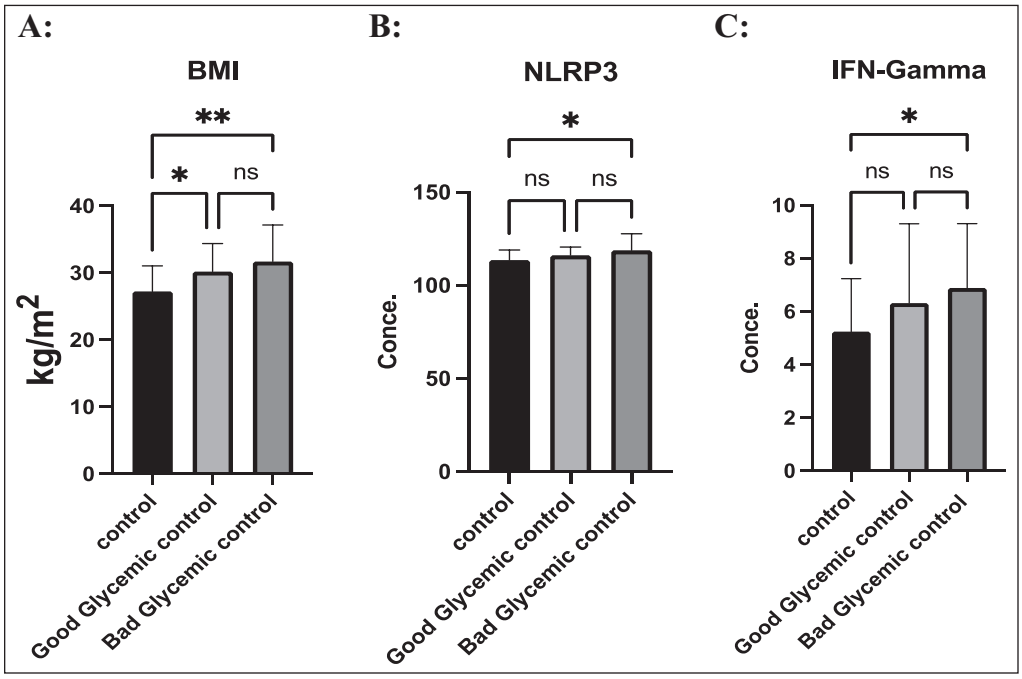

Figure 1: Distribution of investigated parameters in diabetic patient groups (good glycemic control and poor [bad] glycemic control) and control group. (A) BMI means differences calculated by kg/m^2. (B) NLRP3 means differences calculated by pg/mL. (C) IFN-γ means differences calculated by pg/mL. Using Tukey's *post hoc* tests at the (*0.05 and **0.001) levels, there is a significant difference between the independent means

good and bad glycemic control as compared to a control group ($P \leq 0.05$, $P \leq 0.001$), respectively, whereas there was no statistical difference between the two groups of patients.

Serum levels of NLRP3 and IFN-γ only showed a significant increase between the bad glycemic control group of patients and control subjects ($P \leq 0.05$), while this difference was not significant between the good glycemic control group of patients and with either bad control or control subjects as shown in Figure 1B and C.

In the control groups, a significant positive correlation was observed between serum NLRP3 and IFN-γ, as indicated by the correlation with other parameters listed in Table 2 ($P \leq 0.05$). Furthermore, in both good and poor glycemic control, serum NLRP3 exhibited a significant positive correlation exclusively with IFN-γ ($P = 0.0056$ and 0.001, respectively), as shown in Tables 3 and 4.

Table 2: Correlation of serum NLRP3 with BMI, HbA1c, and IFN-γ in the control group

Correlation	NLRP3 versus BMI	NLRP3 versus HbA1c	NLRP3 versus IFN-γ
r	0.02302	−0.2938	0.4638
P (two-tailed)	0.915	0.163	0.022
P value summary	ns	ns	*

* Significant ($P \leq 0.05$) correlation

Table 3: Correlation of serum NLRP3 with BMI, HbA1c, and IFN-γ in type 2 diabetes mellitus with good glycemic control

Correlation	NLRP3 versus BMI	NLRP3 versus HbA1c	NLRP3 versus IFN-γ
r	0.04941	−0.3452	0.5371
P (two-tailed)	0.8146	0.0910	0.0056
P value summary	Ns	ns	*

* Significant ($P \leq 0.05$) correlation

Table 4: Correlation of serum NLRP3 with BMI, HbA1c, and IFN-γ in type 2 diabetes mellitus with poor (bad) glycemic control

Correlation	NLRP3 versus BMI	NLRP3 versus HbA1c	NLRP3 versus IFN-γ
r	0.026	0.071	0.51
P (two-tailed)	0.880	0.681	0.001
P value summary	ns	ns	**

** Significant ($P \leq 0.001$) correlation

Discussion

The complication of T2DM has been shown to be with a big impact on the health care system globally, and vascular damage is the main complication that is conducted consequently by glycation of proteins and lipids caused by prolonged hyperglycemia leading to endothelial dysfunction and stimulation of ROS products leading to stress and inflammation.[10] Monitoring the oxidation markers may be useful in assessing the events in CVD diabetic patients.[11] An increased level of pro-inflammatory cytokine was observed in a study conducted by Al-Tamimi *et al*.,[12] which found that IFN-γ was increased in diabetic subjects with endothelial dysfunction and atherosclerosis. The impact of the glycemic state as defined by HbA1c, in this study was observed after investigating the serum level of NLRP3, and IFN-γ in two diabetic groups with different glycemic control. The mean difference of IFN-γ was assessed and showed statistically increased levels in diabetic patients with poor glycemic control as compared to control subjects while there was no statistical difference between good glycemic control as compared to control subjects. These results are consistence with Mahasa, 2018,[13] which could be explained by the elevated HbA1c caused by prolonged hyperglycemia leading to the inflammatory activity and started by innate immune cells like macrophages as a response to stress leading to CD4 polarization; therefore, IFN-γ production.[14] Consequently, this will lead to increased MHC class I and II expression including beta cells of the pancreas and activation of M1 macrophages of inflammatory effect.[15,16] This activation will progressively contribute to endothelial dysfunction and diabetic complications[17,18]; therefore, studying the factors that could control these events is of huge importance. The study's results suggest that the NLRP3 inflammasome complex may serve as a metabolic danger sensor for the accumulation of high levels of glucose. In other words, when blood sugar levels are poorly controlled in T2DM, there is greater activation of NLRP3, leading to increased inflammation and potentially contributing to endothelial dysfunction.[19] The activation of the NLRP3 inflammasome involves multiple steps and is still an active area of research and one of them is ROS generation in response to hyperglycemia leading to endoplasmic stresses contributing to insulin resistance.[20] This NLRP3 activation could lead to the overproduction of IL-1 β and the infiltration of macrophages in pancreatic beta-cells.[21] Studies on NLRP3 blockade in mice showed that protects against insulin resistance bad effect.[22] Studying the inhibitors of the NLRP3 was conducted by Coll *et al*.[23] who found that an anti-inflammatory therapy could improve T2DM complications. All these findings could support the results of this study, which found a positive correlation between NLRP3 and IFN-γ in the three studied groups suggesting the connecting events in immune response either in healthy or in diabetic subjects. The role of NLRP3 as an indicator of metabolic aberration could be clearly observed by others to create a new effective therapy for patients suffering from metabolic diseases and in the prevention of T2DM complications.

Conclusion

An increased level of NLRP3 was observed in poor glycemic control T2DM and correlated with IFN-γ, suggesting hyperglycemia's effect on this inflammasome pathway that could be associated with aberrant cytokine induction, a key inducer of diabetic complications.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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