Original Article

The Role of the Pro-Inflammatory Cytokine Interferon-Gamma in Type 2 Diabetes and Its Correlation with Atherosclerosis

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Abstract

Background: Immunity plays a major role in the development of atherosclerosis in type 2 diabetes mellitus (T2DM). Pro-inflammatory cytokines, such as interferon gamma (IFN-γ), play an important role in atherosclerosis-related inflammation. **Aims:** This study aims to determine whether IFN-γ level and AIP in patients with T2DM are associated with atherosclerosis development and the effect of IFN-γ concentration level in disease progression. **Materials and Methods:** This study involved 60 Iraqi T2DM patients (30 with dyslipidemia and 30 without dyslipidemia) with an age range of 35–70 years. For the purpose of comparison, 22 healthy participants comparable for T2DM were included in the study. **Results:** this study shows a significant increase in IFN-γ level in the patient groups as compared to the control group ($P < 0.009$). There was a gradualincrease of AIP in the three studied groups with a significant difference ($P < 0.001$). **Conclusion:** The level of IFN-γ and the AIPmay reflect the progression atherosclerosis which may give an alarming sign in studying group patients.

Keywords: Atherosclerosis, interferon gamma, Type 2 diabetes mellitus

Introduction

Diabetes mellitus is linked to a number of microvascular and macrovascular complications, both of which have a significant effect on the global burden of disease mortalities and morbidities.[1] Coronary artery disease, peripheral artery disease, and cerebrovascular disease are all caused by atherosclerosis, which is the most common underlying pathology.[2] The accumulation of vessel-occluding plaques in the subendothelial initial layer of large and medium-sized arteries induces severe stenosis, which reduces blood flow and causes vital tissue hypoxia.[3] The atherogenic index of plasma (AIP) is a new triglyceride (TG) and high-density lipoprotein cholesterol-based index.[4] It is widely used as an optimal predictor of dyslipidemia and related diseases (e.g., cardiovascular diseases).[5] A dyslipidemic condition is common in diabetics with impaired glycemic control, with a rise in TGs, low-density lipoprotein cholesterol (LDL-C), and a decrease in high-density lipoprotein cholesterol (HDL-C).[6] Pro-inflammatory cytokines, such as interferon gamma (IFN-γ), play an important role in atherosclerosis-related inflammation, so inhibiting their development may be beneficial for the anti-inflammatory treatment of atherosclerosis.[7] In this study, we investigate the role of effects of pro-inflammatory cytokines role of IFN-γ in Type 2 diabetes (T2DM) and its correlation with atherosclerosis.

Materials and Methods

A case-control study was conducted on 82 participants, 60 of them were T2DM patients, who were flowed up at the National Diabetes Center\Mustansiriyah University during the period (November 2020 – March 2021), the patients with T2DM were diagnosis according to the American Diabetes Association criteria 2020, and their ages were between 35 and

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70 years. For the purpose of comparison, 22 healthy control participants comparable to diabetes mellitus patients were included in the study. For each participant, 10 ml of blood was collected using the venipuncture method and for the collection of whole blood, plasma, and serum. Commercially, available assay kits were used to assess glucose, HbA1c, total cholesterol, triglyceride, HDL-C levels, and IFN-γ level which was estimated using high sensitivity sandwich ELISA kit (Sunlong Biotech, China) according to manufacturer instructions. The AIP ratio was estimated by the following AIP = Log TG\HDL,[8] while risk ratio-I (RRI) was calculated using the equation of RR-1 = TC\HDL-C, and RR-II (RR-II) = LDL-C\HDL-C.[9]

The data are presented as means standard deviation ANOVA, $P < 0.05$ was considered important. Statistical significance is considered. Was used to compare the differences between the classes. The correlation coefficients between the various studied parameters were determined using the Pearson-analysis year's process. The statistical research was carried out with the aid of the Statistical Package for the Social Sciences SPSS (SPSS V. 25, U.S.A).

Results

The mean of AIP in the studied groups (control, T2DM without and with dyslipidemia) were (0.09 ± 0.54), (0.10 ± 0.07), and (0.39 ± 0.24), respectively, while the means serum levels of IFN-γ in control, T2DM without dyslipidemia, and T2DM with dyslipidemia were (3.62 ± 1.24), (6.25 ± 2.84), and (6.69 ± 2.83), respectively. There was a gradual increase of AIP and IFN-γ in the three studied groups with a significant difference ($P < 0.05$) [Table 1].

The mean ratio of RRI in the control group was (3.18 ± 1.20) and for the T2DM without dyslipidemia was (2.79 ± 0.55), while for T2DM with dyslipidemia was (6.67 ± 4.46). A significant difference was found between the groups as shown in Table 2.

The mean ratio of RRII in control was (2.81 ± 0.54) and for the T2DM without dyslipidemia was (2.91 ± 0.46), while for T2DM with dyslipidemia was (2.21 ± 0.89). A significant difference was found between the groups as shown in Table 2.

The mean cholesterol level of control was (174 ± 25) and for the T2DM without dyslipidemia was (185 ± 23), while for T2DM with dyslipidemia was (158 ± 61). A significant increase difference was found between the groups. The mean triglyceride level of control was (110 ± 25) and the T2DM without dyslipidemia was (114 ± 25), while the mean of T2DM with dyslipidemia was (259 ± 325). Significant increased differences in serum levels were found between the groups. The mean very LDL (VLDL) level of control was (22.09 ± 4.95) and the T2DM without dyslipidemia was (22.99 ± 5.08), while the mean of T2DM with dyslipidemia was (51.97 ± 65.02). A significant increased was found between the groups. The mean HDL level of control was (40 ± 6) and the T2DM without dyslipidemia was (41 ± 3), while the mean of T2DM with dyslipidemia was (35 ± 12). A significant difference was found between the groups. The mean LDL level of the control group was (116 ± 24) and the T2DM without dyslipidemia was (118 ± 21), while the mean of T2DM with dyslipidemia was (79 ± 37). A significant difference was found between the groups [Table 2].

The mean of the diastolic blood pressure of control was (13 ± 3) and the T2DM without dyslipidemia was (14 ± 2), while the mean of T2DM with dyslipidemia was (15 ± 3). Significant differences were found between the groups as shown in Table 3. The mean of the systolic blood pressure of control was (8 ± 1) and the T2DM without dyslipidemia was (8 ± 1), while the mean of T2DM with dyslipidemia was (8 ± 1), no significant differences were found between the groups as shown in Table 3.

As shown in Table 4 the control group, a negative correlation was obvious with cholesterol and RRII, while in T2DM without dyslipidemia a significant positive correlation with (cholesterol, triglyceride, VLDL, and HDL) was observed, and another significant positive correlation with RR-I. In T2DM with dyslipidemia, a positive correlation with (cholesterol, triglyceride, VLDL, and HDL only) can be demonstrated although was not significant.

Discussion

Atherosclerosis is usually considered a chronic inflammatory disease; inflammation plays an important role in all stages of the atherosclerotic process.[10] IFN-γ is one of the key pro-inflammatory cytokines central to many biological processes.[11] The AIP reflects the relationship between protective and atherogenic lipoprotein and is associated with the size of pre- and anti-atherogenic lipoprotein particles.[12]

Diabetic dyslipidemia is a feature of low level of HDL-C, elevated level TG rich lipoproteins, VLDL, and preponderance

Table 1: The mean±standard deviation of atherogenic index of plasma and interferon gamma in the studied groups

Parameter	Mean±SD (range)			*P*
	Control	T2DM without dyslipidemia	T2DM with dyslipidemia	
n	22	30	30	
AIP	0.09±0.54	0.10±0.07	0.39±0.24	0.000**
IFN-γ (pg\ml)	3.62±1.24 (1.63-5.21)	6.25±2.84 (2.68-15.40)	6.69±2.83 (1.10-12.33)	0.009*

*Correlation is significant at the 0.05 level, **Correlation is significant at the 0.001 level. SD: Standard deviation, AIP: Atherogenic index of plasma, IFN-γ: Interferon gamma, T2DM: Type 2 diabetes mellitus

Table 2: Mean±standard deviation level of the risk ratio-I, risk ratio-II, and lipid profile in the studied groups

Parameter	Mean±SD			*P*
	Control	T2DM without dyslipidemia	T2DM with dyslipidemia	
n	22	30	30	
RR-I	3.18±1.20	2.79±0.55	6.67±4.46	0.000**
RR-II	2.81±0.54	2.91±0.46	2.21±0.89	0.000**
Cholesterols (mg\dl)	174±25	185±23	158±61	0.049*
TG (mg\dl)	110±25	114±25	259±325	0.008*
VLDL (mg\dl)	22.09±4.95	22.99±5.08	51.97±65.02	0.008*
HDL (mg\dl)	40±6	41±3	35±12	0.011*
LDL (mg\dl)	116±24	118±21	79±37	0.000**

*Correlation is significant at the 0.05 level,**Correlation is significant at the 0.001 level. RR: Risk ratio, TG: Triglyceride, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, VLDL: Very low-density lipoprotein, SD: Standard deviation, T2DM: Type 2 diabetes mellitus

Table 3: Mean±standard deviation level of the diastolic and systolic blood pressure in control group, Type 2 diabetes mellitus with and without dyslipidemia

Parameter	Mean±SD			*P*
	Control	T2DM without dyslipidemia	T2DM with dyslipidemia	
Number	22	30	30	
Diastolic (mm\Hg)	13±3	14±2	15±3	0.009*
Systolic (mm\Hg)	8±1	8±1	8±1	0.769

*Correlation is significant at the 0.05 level, SD: Standard deviation, T2DM: Type 2 diabetes mellitus

Table 4: Correlation coefficient of serum interferon gamma with a set of parameter among the studied groups

	IFN-γ		
Parameter	Control	T2D without dyslipidemia	T2D with dyslipidemia
FBS	−0.197	−0.254	0.141
HbA1C	−0.257	−0.076	0.056
Cholesterol	−0.523*	0.562**	0.301
TG	−0.127	0.616**	0.248
VLDL	−0.163	0.538**	0.237
HDL	−0.396	0.560**	0.257
LDL	−0.335	0.273	0.122
AIP	0.133	0.296	0.286
RR-I	0.130	0.427*	−0.169
RR-II	−0.553**	0.011	−0.056

*Correlation is significant at the 0.05 level, **Correlation is significant at the 0.01 level. RR: Risk ratio, TG: Triglyceride, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, VLDL: Very low-density lipoprotein, SD: Standard deviation, T2DM: Type 2 diabetes mellitus, AIP: Atherogenic index of plasma, IFN-γ: Interferon gamma, FBS: Fasting blood sugar

of pro-atherogenic small dense LDL.[6] This lipid profile is homely associated with chronic low-grade inflammation and oxidative stress and changes in the plasma level of lipoproteins are known to impact atherosclerosis and associated cardiovascular disease.[13] The present study showed a higher level of total cholesterol in the T2DM patients as compared to control, reflecting the continuous low-grade inflammation that leads to atherosclerosis which is the main complication associated with DM.

The study also showed a significant increase in LDL-C level in the patients' groups as compared to the control group and this elevation and circulatory LDL eventually leads to their accumulation within the arterial wall and increasing their oxidation leading to endothelial dysfunction and facilitates monocytes adhesion to the arterial wall and promotes atherogenesis.[14]

Atherosclerosis index in plasma (AIP) is an important tool for analyzing the lipid profile and is considered an optimal indicator of dyslipidemia and associated diseases,[15] and the association of TG and HDL ratio reflect the balance between risk and protective lipoprotein. AIP provides information about atherogenicity, plasma, and quantifies the response to therapeutic interaction.[13] There was a significant increase in AIP and RRII between the studied groups, this result was in agreement with Viktorinova *et al*., who noticed that the close relationship between AIP and other atherosclerosis-related lipid factors indicates that plasma atherogenicity has increased.[16]

RRI shows a significant difference between the studied groups. This result agreement with Scicali, *et al*. who found that in pre-diabetes and newly diagnosed T2D, TG/HDL is a marker of increased atherosclerotic extension and can be useful in identifying participants with a higher cardiovascular risk profile.[17]

Hypertension (HTN) is often associated with T2DM and people with T2DM will develop HTN.[18] In this study, the result of diastolic blood pressure, increased significantly as compared to the control group which is in agreement with Yannoutsos, *et al*. who found that diabetes is associated with HTN and known to increased cardiovascular disease risk by up to threefold.[19] Other studies conducted by Savin, *et al*. and Butkowski, *et al*. who found that diabetes and HTN disease progression leads to changes in endothelial dysfunction and increased pro-inflammatory and oxidative stress marker and decrease in antioxidant and anti-inflammatory biomarker.[20,21] Atherosclerosis is associated with HTN and is characterized

by oxidative stress and pro-inflammatory activity that may be associated with the observed imbalance between coagulation and fibrinolysis.[22] Atherosclerosis is usually considered a chronic inflammatory disease, and play an important role in all stage of the atherosclerotic process.[10] The inflammatory and oxidative stress can lead to atherosclerosis, accelerated development of arterial thrombosis, and increased risk of death due to CVD.[23]

In the present study, IFN-γ in T2DM without and with dyslipidemia was increased significantly in comparison to the control group and this increase may be due to the sequence of the inflammatory process in which the first line of immune cells natural killers (NKs) and macrophage begin to produce large amount of this cytokine locally as response to the inflammatory action, which may result in recruitment of other inflammatory cells toward the area of plaque formation that may lead to worseness of the action and plaque built up. This result was in agreement with Vivier, *et at*. and Combadière, *et al*. who found in atherosclerotic lesions, cytokines such as IFN-γ induce NK cell migration, activation, cytotoxicity, and also the release more of the pro-atherogenic cytokine IFN-γ. NK cells that express receptors that detect pathological changes or cell stress and are activation of NK, dendritic cells to exhibit potent cell-mediated cytotoxicity through the release of perforin and granzymes, as well as cytokines such as interferon-γ.[24,25] As a result of the continuous inflammatory effect of atherosclerosis on Treg cells become more plastic, which leads to an increase in their number. IFN-γ+Th1/T-regs allowing for more arterial inflammation and atherogenesis.[26] Dong *et al*. found in mice, lowering IFN-γ levels significantly reduce atherosclerotic plaque.[27]

Conclusion

Diabetes is associated with dyslipidemia and atherogenic risks, as well as a rise in the inflammatory cytokine IFN-γ, in stag of pro-atherosclerosis, the T2DM without dyslipidemia-the initial period of atherosclerosis-the level of IFN-γ and the AIP may reflect the progression atherosclerosis which may give an alarming sign and if appropriate treatment is taken, we can prevent this process.

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Conflicts of interest

There are no conflicts of interest.

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