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ORIGINAL ARTICLE

Inflammation and atherogenic markers in patients with type 2 diabetes mellitus☆

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KEYWORDS
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Abstract Type two diabetes mellitus (T2DM) is characterized by a chronic inflammation status. Altered markers such as lipid concentrations are usually found in this disease. Elevated inflammation markers have been described such as cytokines (interleukin 6, tumour necrosis factor-alpha, and IL-8). However, there is a lack of information about the behaviour of the neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), lymphocyte-monocyte ratio (LMR), lipid coefficients, and atherogenic index in T2DM.

Objective: To describe the atherogenic and inflammation parameters in a group of patients with T2DM.

Materials and methods: A total of 42 patients with T2DM were included, all patients were surveyed on clinic history (disease history,comorbidity, smoking, and other relevant variables), measurements of haematological, biochemical,and anthropometric parameters were taken and atherogenic coefficients and inflammation ratios were calculated.

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Results: Inflammation markers such as interleukin 6 and 8, necrosis tumour factor, and NLR were elevated. Of the patients, 88% were classified as high risk according to the atherogenic index. Former smokers had lower levels of IL-8 and higher NLR than non-smokers.
Conclusion: The atherogenic and inflammation markers such as atherogenic index, IL- 8, and NLR make it possible to identify a subgroup of patients that are at risk of severe complications and mortality.

PALABRAS CLAVE
Diabetes mellitus;
Índice aterogénico;
Marcadores de inflamación;
Citocinas;
Índice neutrófilos/linfocitos

Marcadores aterogénicos y de inflamación en pacientes con diabetes mellitus tipo 2

Resumen La diabetes mellitus tipo 2 (DMT2) es una enfermedad caracterizada por un estado inflamatorio crónico en la que algunos marcadores como las concentraciones de lípidos han encontrado frecuentemente alterados. Se han descrito otros marcadores de inflamación alterados como las interleucinas 6, 8 y factor de necrosis tumoral, sin embargo, existe poca información acerca del comportamiento del índice neutrófilo/linfocito (INL), la relación plaquetas/linfocitos (RPL) y el índice monocitos/linfocitos (IML), los coeficientes lipídicos y el índice aterogénico en pacientes con DMT2.
Objetivo: Describir los parámetros aterogénicos y de inflamación en un grupo de pacientes con DM2.
Materiales y métodos: se incluyeron y analizaron los antecedentes clínicos (antecedentes de la enfermedad, comorbilidad, tabaquismo y otras variables relevantes), así como de parámetros hematológicos, bioquímicos y antropométricos de 42 pacientes y se calcularon y evaluaron los coeficientes aterogénicos e índices de inflamación.
Resultados: Se encontraron concentraciones elevadas de citocinas proinflamatorias IL- 6 y IL-8, factor de necrosis tumoral-α y elevado INL. El 88% de los pacientes fueron clasificados como de alto riesgo de acuerdo al índice aterogénico. Los pacientes exfumadores exhibieron niveles menores de IL-8 y niveles más altos de INL comparados con los que nunca han fumado.
Conclusión: La evaluación de marcadores aterogénicos y de inflamación tales como el índice aterogénico, IL-8 y la INL permiten identificar a un subgrupo de pacientes con un alto riesgo de complicaciones graves y mortalidad.

Introduction

Type 2 diabetes mellitus (DMT2) is characterized as a state of chronic inflammation in which immune cellular activation and proinflammatory cytokines play a role in the development, progression and pathogenesis of its complications.[1] Hereditary family characteristics, age and obesity are identifying factors for high risk individuals.[2] Obesity and DMT2 are considered to be states of chronic inflammation, and recently markers such as interleukin 6 (IL-6), tumour necrosis factor alpha (TNF-α) and others such as interleukin 8 (IL-8) are considered to be inflammation markers for these conditions. IL-8 has been linked to DMT2. Patients with DMT2 and obesity have higher levels of IL-8 than non-diabetic subjects.[3–7] IL-8 is produced by phagocytes and mesenchymal cells exposed to inflammatory stimuli (IL-1 or TNF-α) which activates neutrophils and induces chemotaxis, exocytosis and oxidative burst. Nicotine has recently been reported to be able to induce IL-8 secretion.[8] Several haematological parameters are affected in subjects who smoke, and the role of smoking among the causes of pathogenesis of diabetes and cardiovascular disease has been underlined. Nevertheless, since 1992 evidence has emerged that after ceasing to smoke these alterations are modified to levels similar to those observed in non-smokers.[9]

It has recently been shown that changes were detected in the levels of expression of 119 genes in the 8 weeks after ceasing to smoke. This includes the expression of certain genes which are associated with cytokines, and these changes occurred in the first 4 weeks after cessation.[10] Ceasing to smoke reduce the risk of several chronic diseases, and it is associated with increased life expectancy. Smoking is associated with the loss of a decade of life and stopping smoking reduces this loss by up to 90%. Smokers who cease to smoke from the age of 45–54 years old and those who do so from 55 to 64 years old recover from 6 to 4 years of life, respectively.[11]

Reducing smoking has also been associated with a reduction in the concentrations of leucocytes, erythrocytes, haemoglobin, haematocrit and fibrinogen, as well as a significant increase HDL levels and the HDL/LDL coefficient.[12]

Leukocytes are important predictors of several diseases, and some of their subtypes, such as the total count of

monocytes and lymphocytes are also long-term predictors of mortality due to a range of diseases.[3]

Markers such as the neutrophil/lymphocyte ratio (NLR) and the platelet/lymphocytes ratio (PLR) are indicators of subclinical inflammation, and some studies have reported high level in patients with DMT2.[13] The lymphocyte/monocytes ratio (LMR) has been proposed as an endothelial dysfunction and inflammation marker in different study populations, and it has been assigned a prognostic value.[3]

Another marker, the atherogenic index, has been linked to high concentrations of reactive C protein in patients with acute coronary syndrome, and it may probably be linked to others such as NLR, LMR, and PLR.[14] The atherogenic index has been shown to be associated with atherosclerosis in the general population,[15] and as a whole with haematological and immunological markers these may be risk and predictive markers for major complications in obese patients with DMT2.

Objective

To describe the atherogenic and inflammation parameters in a group of patients with DMT2.

Design

The data were analysed using a survey design.

Patients and methods

A total of 42 patients with a diagnosis of DMT2 were included. The study was carried out in a multidisciplinary clinic for the treatment of patients with chronic non-transmissible diseases in the city of Lerdo, Durango, Mexico, a small semi-urban region of approximately 80,000 inhabitants. The clinic has 100 patients with chronic degenerative diseases, of whom 59% have diabetes. The inclusion criteria were as follows: having been diagnosed DMT2, having a reference to be treated in the clinic, not having acute inflammation, an infectious or autoimmune disease, having accepted to take part in the study by signing the informed consent document. Patients who did not attend their appointment for laboratory studies were excluded.

All of the participants were asked about the following variables: hereditary family history, age, sex, smoking and other comorbidities. They were measured and their body mass ratio was calculated (BMI, kg/m^2), and the circumference of their waist and hip were measured (cm) using a flexible optic fibre tape while they were lightly dressed. Their waist circumference was measured at the level of the smallest circumference between the iliac crest and the thoracic cavity. Hip circumference was measured at the level of the largest protuberance of the iliac crest, and the waist/hip ratio was calculated. Smoking as classified as ''never'' if the subjects stated that they had never smoked, and as ''ex-smoker'' if they stated that they had ceased to smoke at least 2 weeks before enrolling in the study. Ex-smokers were classified as light smokers (if they smoked $\leq$10 cigarettes per day) and as moderate/heavy smokers if they smoked >10 cigarettes per day) according to the classification reported by Pulvers et al.[16] No subject stated that they were a smoker at the time of the study.

All of the patients were given an appointment in the clinic during the morning while fasting, and a blood sample was taken for a complete blood count (neutrophils, lymphocytes, monocytes and platelets), biochemical parameters (lipid profile) and immunological parameters (concentrations of IL-6, IL-8 and TNF-α).

The atherogenic index was calculated (LogTG/HDL), together with several coefficients such as the low-density lipoprotein/high density lipoprotein ratio (LDL/HDL), total cholesterol/high density lipoprotein (TC/HDL) and triglycerides/high density lipoprotein (TG/HDL). Following previous studies, the atherogenic index was classified as low risk if the score was <0.11, medium risk at 0.11—0.21 and high risk at >0.21.[17,18]

The ELISA (enzyme-linked immunosorbent assay) method was used to measure cytokines, and commercial reagents were used following the manufacturer's (Abcam) instructions. The assays were performed in duplicate on 96-well plates, and the results were expressed in pictograms per decilitre. The detection range for IL-6, IL-8 and TNF-α was from 1.56 to 50 pg/mL, 31.25 to 1,000 pg/mL and 25–800 pg/mL, respectively; the smallest detectable doses (SDD) were lower than 0.81, 0.29 and 8 pg/mL, respectively.

Statistical analysis

Quantitative variables are expressed as an arithmetical mean and standard deviation (SD), and qualitative variables are shown as frequencies and percentages. Groups were compared for immunological variables and atherogenic parameters using the Student's t-test or Mann–Whitney's U test, depending on their distribution. The level of statistical significance was set at $P < .05$. The statistical package SPSS 23.0 was used for data analysis.

Ethical considerations

This study was approved by the Ethics and Research Committee of Hospital General de Gómez Palacio, Durango, and by the Research Committee of the Health Science Faculty of Universidad Juárez del Estado de Durango. It was conducted under the regulations and principles of the Helsinki Declaration. All of the participants in the study gave their informed consent before they were included.

Results

Seventeen of the 59 patients with DMT2 were excluded because they did not attend their appointment for laboratory studies. Of the 42 patients who were included, 27 (64.3%) were women. The average age of the group was 55.48 $\pm$ 11.05 years. 52.3% of the patients lived in rural areas; 26 (61.9%) had at least one comorbidity; 52.4% of the patients had hypertension and 35.7% had dyslipidaemia.

Forty-two-point nine percent of the patients were overweight, 19% had grade I obesity and 7.1% had grade II obesity. 83.3% of the population had an increased waist/hip ratio

Table 1 Anthropometric and haematological parameters of the patients.

Anthropometric parameters	n = 42	Haematological parameters	n = 42
Weight (kg)	71.98 ± 16.97	Leukocytes (u/μL)	7.67 ± 1.61
Height (m)	1.61 ± .10	Haemoglobin (g/dL)	13.79 ± 1.84
BMI (kg/m^2)	27.48 ± 5.42	Platelets (u/μL)	271.67 ± 79.82
Waist circumference (cm)	98.90 ± 9.53	NLR	2.50 ± 1.52
Hip circumference (cm)	104.32 ± 12.54	LMR	5.74 ± 2.21
Waist-hip ratio	.95 ± .80	PLR	10.80 ± 9.15

All data are expressed as averages and standard deviation.
BMI, body mass index; MLR, monocyte/lymphocyte ratio; NLR, neutrophil/lymphocyte ratio; PLR, platelet/lymphocyte ratio.

(greater than 0.85 and 0.94 for women and men, respectively). 42.9% of the subjects were ex-smokers (11 men and 7 women) and their average daily consumption of cigarettes per patient was 8.6 per day; 14 (77.8%) of the patients were light smokers and 4 (22.2%) were moderate to heavy smokers. The anthropological and haematological data of the subjects are shown in Table 1.

Levels in serum of IL-8 were 63.88 ± 32.36 pg/mL, 13.07 ± 9.61 pg/mL for IL-6, and for TNF-α they were 745.11 ± 864.60 pg/mL. Biochemical data and the total atherogenic risk coefficients of the patients are shown in Table 2.

Eight (19%) of the total number of patients with dyslipidaemia were taking lipid lowering treatment. The calculated atherogenic risk was high, and the TC/HDL coefficient corresponded to medium risk for the majority of the patients; these data are shown in Table 3.

We compared the parameters of the inflammatory and atherogenic markers according to BMI category, the waist-hip ratio and sex, without finding statistical differences. However, in the comparison based on smoking significant differences were found in the total number of platelets, percentage of lymphocytes and levels of HDL, NLR and IL-8 (Table 4).

The subjects were then divided into two groups based on their median waist circumference, and we compared all of the parameters for both groups. Statistically significant differences were found between the group with a waist measurement greater than the median and the group with a measurement less than the same. This was so for IL-8 levels (65.65 vs. 53.42 pg/dL, $P = .01$), the cholesterol/HDL ratio (3.98 vs. 4.74, $P = .049$) and there was also a tendency towards statistical significance in the total leukocyte count (7.71 vs. 7.38, $P = .056$) and for PLR (9.21 vs. 8.74, $P = .07$).

The same comparisons above and below the median were made afterwards in the non-smokers group, and differences were found in triglycerides, HDL, VLDL and the atherogenic index (Table 5), while no statistically significant differences were found in the group of ex-smokers.

Discussion

The results of this study show that 69% of the patients included in it are overweight or have some degree of obesity. 42.9% of the patients are ex-smokers and 73.8% had a family history of diabetes mellitus. Few of the patients had the illness under control; the average HbA1c was 9.35%, meaning that this population has poor control of the disease, and this may be linked to adipose tissue remodelling due to inflammation and insulin resistance. The literature reports that lack of control of the disease permits the general activation of the innate immune system.[19,20]

The immune response, genetic predisposition and the obesogenic environment are associated with increased bodyweight, which causes adipose tissue dysfunction, the infiltration of macrophages and an increase in the secretion of cytokines such as IL-6 and TNF-α.[21]

High concentrations of IL-6, IL-8 and TNF-α were found in all of the patients in comparison with the levels reported by other authors in healthy subjects and patients with DMT2.[7,22–24] Different authors have shown that poor control of glucose and the resulting hyperglycaemia induce the glycosylation of molecules such as proteins and lipids. The binding of these glycosylated molecules to their receptors in the innate immune system cells, particularly to monocytes/macrophages and neutrophils, is associated with the altered functioning of these cells.[25]

Chronically high levels of specific inflammatory markers such as IL-6 and TNF-α seem to be involved in metabolic disorders.[26] Overproduction of them induces the hepatic synthesis of reactive C protein and promotes the start of cardiovascular complications, altering insulin sensitivity by modifying different steps in the insulin signalling pathway, thereby perpetuating resistance to the same.[27,28]

The different inflammation and atherogenic markers were compared between the BMI and waist-hip ratio categories without finding any differences. However, when the comparison was made according to smoking history, higher levels of IL-8 were found in non-smoking subjects compared to the ex-smokers. This result is of interest given that nicotine has previously been reported to induce the secretion of IL-8 in patients who are smokers.[8] Given this and based on the above-mentioned findings we believe that high levels of IL-8 when smoking may have returned to basal levels when smoking ceased, and that a bias in exposure favoured an alternative change in lifestyle that may be associated with our findings.

Studies on reversion of the harm caused by smoking after cessation show a reduction in the levels of total cholesterol, LDL and fibrinogen. A relationship has also been shown to exist between the total leukocyte count and the incidence of coronary disease and vascular accidents. In the same way, stopping smoking has bene associated with an improvement in established cardiovascular risk factors, including the levels of haemoglobin, fibrinogen, erythrocytes and leukocytes.

Table 2 Biochemical and atherogenic characteristics of the patients.

Biochemical profile	n = 42	Biochemical profile	n = 42
Glucose (mg/dL)	177.57 ± 67.09	HDL (mg/dL)	50.86 ± 17.50
Urea (mg/dL)	35.68 ± 15.52	LDL (mg/dL)	99.52 ± 33.34
Creatinine (mg/dL)	.96 ± .43	VLDL (mg/dL)	50.98 ± 32.96
Glycosylated haemoglobin (%)	9.35 ± 2.68	LDL/HDL coefficient	2.09 ± .81
Cholesterol (mg/dL)	201.83 ± 40.77	TC/HDL coefficient	4.24 ± 1.21
Triglycerides (mg/dL)	256.29 ± 167.42	LogTG/HDL	.64 ± .31

All data are expressed as the arithmetic average and standard deviation.
HDL, high density lipoproteins; LDL, low density lipoproteins; LogTG/HDL, atherogenic index; VLDL, very low density lipoproteins.

Table 3 Atherogenic risk in patients with DMT2.

	Low risk n (%)	Medium risk n (%)	High risk n (%)
LDL/HDL coefficient	38 (90.5)	2 (4.8)	2 (4.8)
TC/HDL coefficient	17 (40.5)	25 (59.5)	–
LogTG/HDL	3 (7.1)	2 (4.8)	37 (88.1)

n = 42 subjects. All data are expressed in frequencies and percentages.
LogTG/HDL, atherogenic index.

Table 4 Haematological, biochemical and immunological parameters of the ex-smoker and non-smoker patients.

Parameters	Non-smokersn = 24Median (ranges)	Ex-smokersn = 18Median (ranges)	p
Platelets, u/dL	303.11 (271.69—457.62)	270.35 (189—271.25)	.05
Lymphocytes, %	32.53 (29.88—44.39)	29.55 (20.60—30.68	.05
HDL, mg/dL	55.67 (50—85.66)	45.50 (38.25—50.00)	.04
IL-8, pg/dL	72.08 (63.96—84.15)	54.10 (37.28—62.42)	.01
NLR	1.97 (1.60—2.56)	2.56 (1.90—3.35)	.04

The Mann–Whitney U test was used for analysis. A value of $P \leq .05$ was considered to be statistically significant.

Table 5 Biochemical and atherogenic characteristics of non-smoker diabetic patients according to waist circumference.

Biochemical profile	Non-smokers n = 24	Non-smokers with a low waist circumference n = 16	Non-smokers with a high waist circumference n = 8	p
Triglycerides (mg/dL)	228 (154.3—342)	179 (140.5—294.8)	317 (236.8—652.8)	.043
VLDL (mg/dL)	48.50 (33—68.75)	37.50 (28.5—58.5)	63.50 (47.5—130.5)	.049
LogTG/HDL	0.67 (0.41—0.92)	0.50 (0.35—0.79)	0.94 (0.66—1.16)	.040
Cholesterol/HDL	4.06 (3.15—4.75)	3.69 (3.06—4.31)	4.88 (4.12—6.05)	.029

Data are expressed as medians and ranges. The Mann–Whitney U test was used for comparison and a value of $P \leq .05$ was considered to be a statistical difference.

The dose-response effect of smoking on haematological parameters confirms the principle that reducing exposure has beneficial effects at an individual level as well as for public health.[10–12]

The NLR was high, confirming subclinical inflammation in our patients. However, when the comparisons were made according to smoking history, the NLR was higher in ex-smokers than it was in non-smokers; this indicates that stopping smoking has many benefits, although the presence of other factors, such as the high atherogenic index, alterations in the lipid profile and poor control of diabetes continue to generate the state of chronic inflammation. These markers, the NLR and the atherogenic index may therefore be studied and used as predictors of morbidity and complications in patients of this type.

The World Health Organization Compendium of Indicators (2015) of the Framework Agreement for the Control of Tobacco, and the National Survey of Health Interviews of the Centre for Disease Control and Prevention (August 2017) define ex-smokers as those adults who have smoked at least 100 cigarettes in their lives but had ceased to smoke at the time of the interview. Bain et al. (1992), Sunyer et al. (1996) and Roethig et al. (2010) demonstrated an improvement in haematological factors such as the neutrophil, lymphocyte and platelet counts only 2 weeks after having ceased smoking.[9] They prove that subjects who reduce cigarette consumption show changes in the leukocyte count during the first 6 months after changing their practice[29] and that the reduction in exposure to cigarette smoke suggests a reduction in inflammation. Switching from conventional to electronic cigarettes or stopping smoking led to a fall in leukocytes and vice versa in 3 days.[30] According to these data, we define ex-smokers as subjects who had ceased to smoke at least 2 weeks prior to their inclusion in the study.

Although we are not aware of the parameters of the group of ex-smokers before they ceased smoking, we suggest that all of their altered markers improved substantially after they had stopped smoking.

According to the publication by Donath and Shoelsonen in 2011, immune system components are altered in cases of obesity and DMT2. The majority of these changes occur in the adipose tissue, the liver, pancreatic islets, the vasculature and circulating leukocytes.[31] Changes in the adipose tissue and other locations are associated with other markers, such as levels of triglycerides, HDL, LDL, VLDL, cholesterol and atherogenic markers such as the LDL/HDL, VLDL/HDL and TC/HDL coefficients and the atherogenic index (LogTG/HDL).

The atherogenic markers in these patients were classified as low risk (>3.0 and <4) for the LDL/HDL coefficient, and as medium risk (>4 to <7) for the TC/HDL coefficient.[32] The average value of the atherogenic index was 0.64, which is considered to be high risk. However, the coefficients do not show this in the same way, and this may be due to the small number of patients (19%) who were receiving lipid-lowering treatment. Furthermore, our total population had a larger waist circumference and a greater BMI. The non-smoker subjects above the median waist circumference showed higher levels of triglycerides and an atherogenic index that was higher than it was for those subjects who had a waist circumference smaller than the median value. According to the report by Li et al., waist circumference and BMI correlate directly with atherogenic risk,[33] and this is reflected in our results.

This is an alarming situation in our patients, due to the increased risk of cardiovascular disease and the associated increase in mortality. In our country cardiovascular disease occupies the first places in the causes of mortality, and our patients are at high risk of suffering an associated event, so that it is a priority to confirm these findings by other studies, determining the cause of the failures and creating new prevention and treatment programmes.

As the NLR and atherogenic index are low cost and easy to obtain, they could be used as chronic inflammation markers, cardiovascular disease predictors and predictors of increased mortality and the risk of complications in diabetic and obese patients. Haematological indexes such as the lipid profile are analytical data which may be found for all of the patients who consult, and they may be associated with micro- and macro-vascular complications, as other authors have published.[34–36]

Atherogenic and inflammation markers such as the atherogenic index, IL-8 and the NLR may aid in the identification of a subgroup of high-risk patients for severe complications. It is very important act urgently and treat these patients as a group at moderate to high cardiovascular risk, according to the recommendations of the ''Guide in diabetes mellitus, prediabetes and cardiovascular diseases'',[37] ensuring that they make lifestyle changes, including smoking cessation, weight loss, controlling their glucose, improving their glycosylated haemoglobin levels, controlling comorbidities and ensuring adherence to treatment to prevent complications. The effects of advanced age and comorbidities show the need to manage risk on an individual basis, empowering patients so that they themselves control their disease.

This is the first study to evaluate the state of atherogenic and immunological markers in patients with DMT2 in a small region of the State of Durango, Mexico, and due to its exploratory nature it has limitations intrinsic to the sample size and its cross-sectional design. However, we found significant differences in NLR and IL-8 values between the group of non-smokers and ex-smokers in the atherogenic index when their values were compared above and below the median waist circumference. These findings are reproducible and applicable, especially in patients with DMT2 and those less than 40 years old. They require confirmation using longitudinal designs to evaluate the usefulness of biomarkers in patients of this type, as well as their utility as predictors of complications.

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Conflict of interests

The authors have no conflict of interests to declare.

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