

Exploration of the Relationship Between Interleukin 37 and Folic Acid in Type 2 Diabetic Patients

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ABSTRACT

Background: Type 2 diabetes (T2DM) It is a complex disease associated with metabolic disorders. The main aims of the study was to accurately measure the concentration of interleukin (IL-37) and folic acid and explore the possible association between them in T2DM patients and compare it with healthy controls (HCs).

Materials and Methods: Identical in gender and age, 72 diabetics and 16 healthy controls participated in this study; IL-37 serum concentration was estimated by ELISA technique, serum levels of folic acid by electrochemiluminescence method.

Results: An IL-37 serum concentration was significantly greater and folic acid serum concentrations lower levels in patients with T2DM compared to HCs, while urea and creatinine didn't show significant changes between studied groups. There was strong negative relationship among serum IL-37 and folic acid levels in T2DM. but urea and creatinine was presented non-significant association with IL-37 ($p > 0.05$), however they exhibited a significant association with folic acid ($p < 0.05$). There was no significant relationship of gender with serum IL-37 & folic acid (both $p > 0.05$) in patient groups and HCs. Area under curve from Receiver operating characteristic (ROC) analysis, was shown that Interleukin 37 has the highest value compared to all the studied biomarkers.

Conclusion: This study demonstrated the strong negative correlation between IL-37 and folic acid and the important role for them in predicting and monitoring disease and possibly in the treatment of type 2 diabetes patients.

Keywords: Interleukin-37, Folic Acid, T2DM, HbA1c.

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INTRODUCTION

Diabetes is defined from a medical perspective as the partial or total deficiency of insulin that leads to hyperglycaemia and is defined from a social perspective as the burden imposed by the disease on the economics in terms of the cost of treatment, complications and death rate, and from the patient's perspective it is defined as a condition that requires daily attention to diet, lifestyle and permanent use for treatment, and constant monitoring of level blood sugar throughout life, with anxiety and depression [1]. Conventionally, the injurious penalties of hyperglycemia are labeled into two components specifically macrovascular problems and microvascular complications. The microvascular issues consist of retinopathy, diabetic nephropathy, and neuropathy whereas, the macrovascular issues encompass stroke, coronary artery sickness and peripheral arterial disorder [2]. On the basis of the disease-causing measures that lead to hyperglycemia, the disease is classified. The first and second types are broad classifications, followed by other types, including gestational diabetes and other types due to congenital diabetes due to genetic defects in insulin secretion or associated with cystic fibrosis, and steroid diabetes resulting from glucocorticoid overdoses [3]. Type 2 diabetes mellitus (T2DM) It is a metabolic complex disease associated with a defect in the metabolism of fats, proteins and carbohydrates, and is greatly affected by very complex interactions with environmental and genetic factors. [4] because it is characterized by chronic hyperglycemia and a pro-inflammatory metabolic

disorder, it will lead to an elevated level of cytokines circulating in patients, indicating a causal role of inflammation in its etiology [5]. Cytokines are important mediators of immune and inflammatory immune responses [6]. Interleukin-37 (IL-37) is a new member of the family IL-1, expressed in a variety of cancers, autoimmune and inflammatory disorders and that it has an important vital role in inhibiting the adaptive and innate immunity response and inflammatory reactions [7]. and in many human tissues it has been expressed, including the colon, placenta, esophagus, breast, prostate, tonsils, and skin [8]. In Iraq, there are several studies on Interleukin 37, but most of them are with rheumatoid arthritis, the most recent by Moslima *et al* (2020) [9]. Vitamins are a group of nutrients in health and play a very important role in health. These chemicals are involved in many physiological and functions and endanger health with their deficiency or spare [10]. Folic acid additionally acknowledged as vitamin B9, is biocompatible, existing in foods and take on as a dietary supplement [11]. It is essential, water-soluble nutrients that have an main part in the growth, directive of cell division over its role in DNA synthesis, methylation, repair and amino acid metabolism [12]. Main goals of this study were evaluate serum IL-37 & Folic acid concentrations as well as their correlations with each other in T2DM

Materials and Methods

In this study 72 patients with T2DM, age ranged from 25 to 65 years. This study was conducted during in the

period from December 2019 to March 2020. After clinical examination by the consultant physicians and after approval from the patients, Samples were collected; the history of treatment and lifestyle has been taken into account After 8 hours of fasting, 10 ml were taken from 9 am to 11 am and divided into sections using a 10 ml syringe intravenously from each healthy and sick person. The first part was distributed in a tube containing tetraethylene acetic acid to estimate HbA1c on the same day, At room temperature, the other part was left for coagulation and for a period of a quarter of an hour, the serum was expelled at 5000 xg for use in assessing blood glucose, S.creatinine, S.urea, and in the last part, the serum was taken after separation and a volume of (200µl) was taken from it for later use and stored in the freezer (-20°C) to estimate the concentration of interleukin and what was left for the purpose of estimating Folic acid. The serum IL-37 was measured by Enzyme Linked Immune-Sorbent Assay (ELISA), the ELISA kits providing by (Al-Shkairate establishments, Jordan) and Serum levels of Folic acid was estimated by Electrochemiluminescence method kits provided by (Roche, Germany) and by colorimetric reactions, serum levels of urea, creatinine (Linear, Spain) and glucose (Biolabo, France) were estimated, hemoglobin levels was estimated by sandwich immune detection method (Boditech, Korea). t. They were compared with 16 healthy controls, who were also aged from 25 to 65 years.

Statistical analysis

Using GraphPad prism version 7.04 and Software Excel 2016, Statistical analysis of the data was done. The descriptive statistics for each parameter consisted of the standard error (SE), the mean and the standard deviation (SD). The level of statistical significance was set as long as P value fewer than 0.05, the relations among IL-37 and Folic acid features of together T2DM Patients and healthy control was calculated through Pearson’s correlation (r=-1 to 1), a receiver operating characteristic (ROC) curve was formed to test the levels of IL-37 and folic acid among patients T2DM with HCs.

Results

In Table (1) The results were obtained in this study as following, significant increase IL-37 serum levels in patients than compare to healthy control there was significant different between Two group. on the other hand, Mean of Folic acid was decreased significantly in patients compare with healthy control. Non-significant difference was noticed in serum levels of urea and creatinine between both groups and also Age, but Serum glucose levels were increased significantly in patients compare with healthy control, also HbA1c and mean of BMI, WHR in patients increase In comparison with healthy control.

Table (1): Mean and S.D in Patients and HCs

Parameter	Healthy Controls			T2DM Patients			
	Mean	SD	SEM	Mean	SD	SEM	p-value
Age years	45.88	12.25	3.062	50.17	11.51	1.357	0.1859
WHR	0.77	0.06947	0.01737	1.04	0.2377	0.02802	<0.0001
BMI kg/m ²	22.71	2.237	0.5591	25.67	4.023	0.4741	0.0056
FSG mg/dL	111.3	48.9	12.23	199	94.13	11.09	0.0005
HbA1c %	5.353	0.6759	0.169	8.926	2.469	0.2909	<0.0001
Creatinine mg/dL	0.7688	0.1957	0.04892	0.9389	0.4244	0.05002	0.1221
Urea mg/dL	31.03	9.838	2.46	38.06	16.37	1.929	0.1033
IL-37 pg/mL	497.4	178.9	44.73	1390	403.2	47.51	<0.0001
Folic Acid ng/mL	12.02	4.157	1.039	7.179	3.797	0.4475	<0.0001

In Table (2) The Correlation between IL-37 with folic acid was strong negative. Correlation of IL-37 with WHR, BMI and HbA1c was strong positive (P <0.01) with(r = -0.392, r = 0.503, r = 0.525 and r=0.322 Fig. 1, 2, 3, and 4 respectively), While there is a weak positive correlation between IL-37 with FSG at P <0.05 (r = 0.245) (Fig. 5) But non correlation was noticed between IL-37 with age,

creatinine and urea. According to Pearson Correlation Analysis Table (2), strong negative correlation was noticed between Folic acid with IL-37, also with creatinine and with urea (r =-0.392, r = -0.248, r = -0.318 at P <0.01, Fig. 6, 7 and 8 respectively) and non-correlation between folic acid with F.S.G, age, WHR, BMI, and HbA1c.

Table (2): Correlation between IL- 37 and Folic acid with Some Variables in all Patients of T2DM

Parameter	IL-37 pg/mL	Folic acid ng/mL	Age years	WHR	BMI kg/m ²	FSG mg/dL	HbA1c %	Creatinine mg/dL	Urea mg/dL	
IL-37 pg/mL	r	1	-0.392	0.141	0.503	0.525	0.245	0.322	0.139	0.073
	P		0.0002	0.1888	<0.0001	<0.0001	0.0214	0.0023	0.1957	0.5017
Folic acid	r	-0.392	1	-0.128	-0.185	-0.175	-0.212	-0.179	-0.248	-0.318
	P	0.000		0.2357	0.0843	0.1033	0.0478	0.0959	0.0196	0.0025

ng/mL		2							
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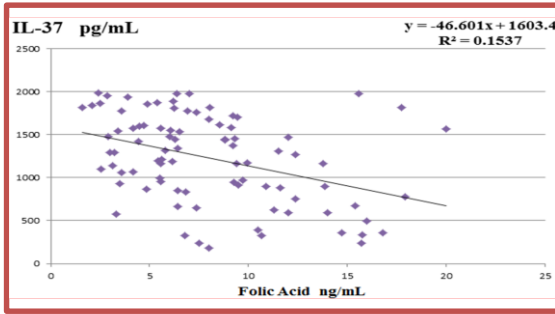


Fig. (1): Correlation between IL-37 with folic acid

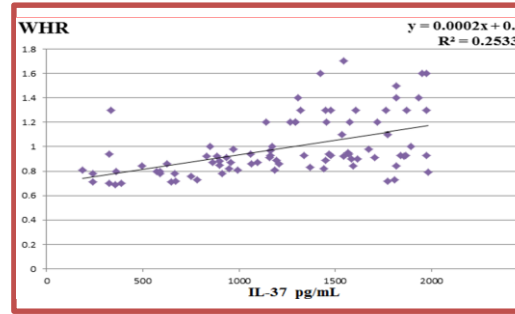


Fig. (2): Correlation between IL-37 with WHR

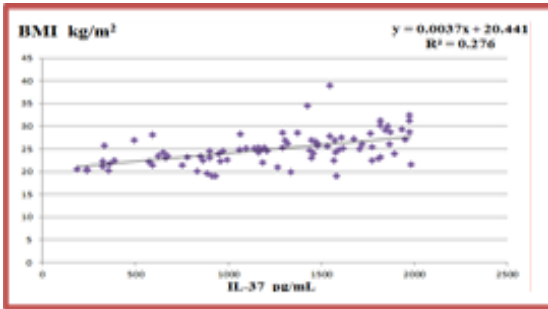


Fig. (3): Correlation between IL-37 with BMI

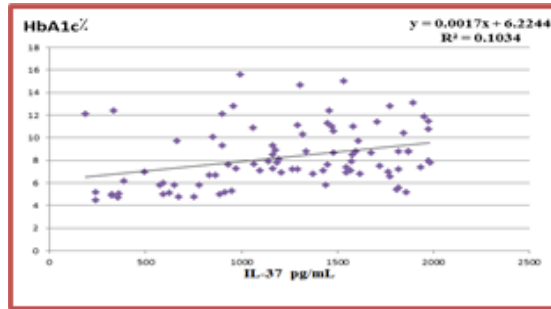


Fig. (4): Correlation between IL-37 with HbA1c

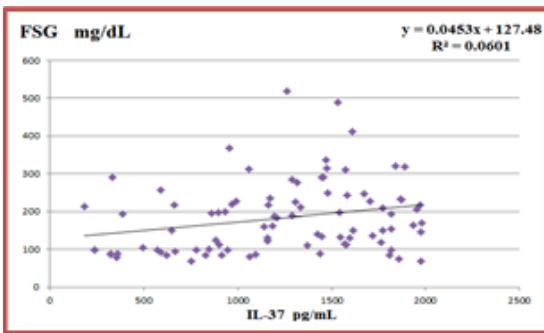


Fig. (5): Correlation between IL-37 with FSG

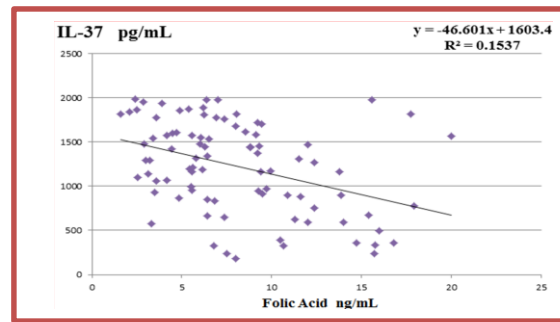


Fig. (6): Correlation between Folic acid with IL-37

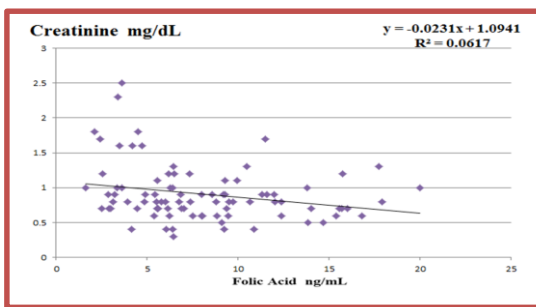


Fig. (7): Correlation between Folic acid with Creatinine

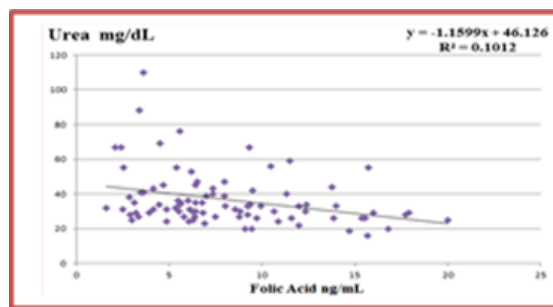


Fig. (8): Correlation between Folic acid with Urea

As Table (3) shows, the ROC curve recognized that the serum IL-37 was among the standards with the highest validity, and provides an almost perfect test with exhibited an excellent and fantastic a way to distinguish between sick and healthy individuals DM [AUC = 0.9731; P < 0.0001; 95% Confidence Interval (CI): 0.9387 to 1.007 and SE: 0.01753] (Fig. 9), while HbA1c %value is a very important parameter with a value of [AUC = 0.954; P < 0.0001; 95% CI: 0.9131 to 0.9949 and SE: 0.02087] (Fig. 10), it provides an ideal test as well. and also was found WHR [AUC = 0.9214; P < 0.0001; 95% CI: 0.8492 to

0.9937 and SE: 0.03686] (Fig. 11), as well FSG it has been found to be a better predictor of T2DM than Folic acid [AUC = 0.8181; P = <0.0001; 95% CI: 0.7049 to 0.9313 and SE: 0.05775] vs [AUC = 0.8151; P = <0.0001; 95% CI: 0.6961 to 0.9341 and SE: 0.0607] respectively (Fig. 12 and 13) In addition, it can be stated that BMI index value constitutes data for disease risk with [AUC = 0.7609; P:0.0012; 95% CI:0.6387 to 0.883 and SE:0.06231] (Fig. 14), Finally, age , Urea and Creatinine were among the parameters that provided the lowest validity in predicting the T2DM.

Table (3): Area under the ROC curve for all analyzed Biomarkers				
Parameter	AUC	Std. Error	95% confidence interval	p-value
Age years	0.6076	0.07457	0.4615 to 0.7538	0.1798
WHR	0.9214	0.03686	0.8492 to 0.9937	<0.0001
BMI kg/m2	0.7609	0.06231	0.6387 to 0.883	0.0012
FSG mg/dL	0.8181	0.05775	0.7049 to 0.9313	<0.0001
HbA1c %	0.954	0.02087	0.9131 to 0.9949	<0.0001
Creatinine mg/dL	0.6241	0.06782	0.4912 to 0.7571	0.1219
Urea mg/dL	0.6194	0.07602	0.4704 to 0.7683	0.1369
IL-37 pg/mL	0.9731	0.01753	0.9387 to 1.007	<0.0001
Folic Acid ng/mL	0.8151	0.0607	0.6961 to 0.9341	<0.0001

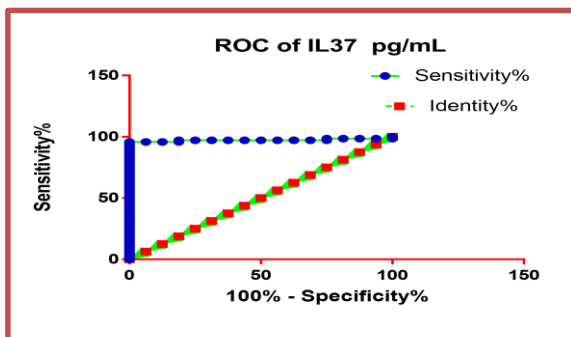


Fig. (9): ROC curve showing AUC of IL-37 in T2DM

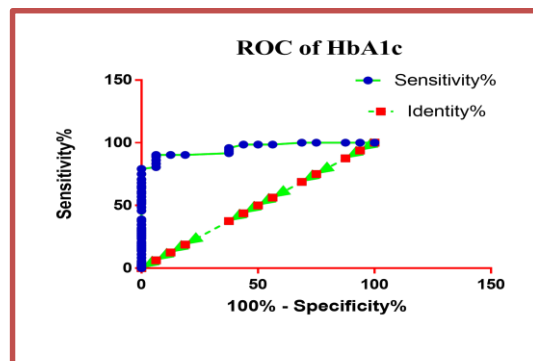


Fig. (10): ROC curve showing AUC of HbA1c in T2DM

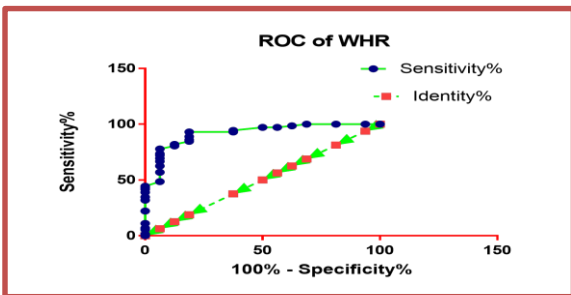


Fig. (11): ROC curve showing AUC of WHR in T2DM

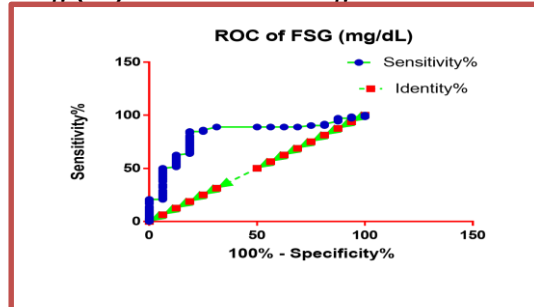


Fig. (12): ROC curve showing AUC of FGS in T2DM

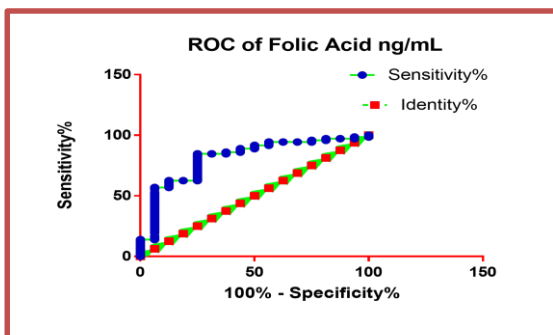


Fig. (13): ROC curve showing AUC of Folic acid in T2DM

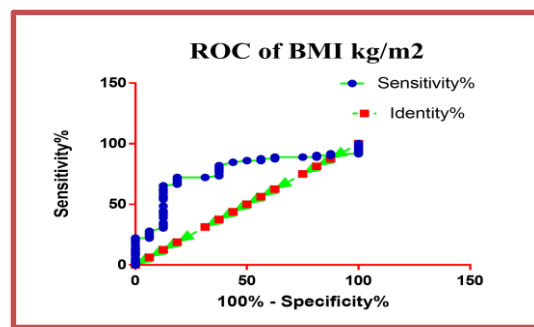


Fig. (14): ROC curve showing AUC of MBI in T2DM

Discussion

Type 2 diabetes mellitus (T2DM) is a chronic inflammatory disease enhanced by modifications in cells to immunity and T cells are considered mediators and important for inflammation [13]. IL-37 is a new member of the family IL-1, with an extra and greater rising function in every innate and adaptive immune

responses, IL-37 is the fully considered, It is anti-inflammatory and the property it shows is widespread [14]. and that IL-37 is usually expressed in granule cells and T cells, with the best degree of statement in regulatory T cells (Treg) [15]. Therefore, IL-37 stages are abnormal in sufferers with inflammatory and autoimmune diseases, which include sickness Diabetes

[16]. Our results were consistent with the results of one of the studies that found an increase in the value of interleukin 37 in elderly mice with type 2 diabetes [17]. Folic acid is a bioactive food aspect whose deficiency can lead to a range of fitness problems, whilst a excessive consumption of folic acid can decrease the cytotoxicity of natural killer cells [18]. The high concentration of homocysteine is associated with many health problems, and it is an amino acid that is contrariwise linked to folic acid. It has been observed that deficiency of these vitamins occurs relatively regularly in the developing world, especially in Pakistan. Several studies have noted an association with stages of excessive homocysteine and diabetes, so homocysteine could be an indicator of folic acid (vitamin B9). And studies conducted on patients with type 2 diabetes that hyperhomocysteinemia is associated with folic acid and the consumption of this vitamin to manage and reduce homocysteine. Therefore, attainable approach to manage plasma Hcy is on hand in the shape of these vitamins [19]. The metabolism of methionine and homocysteine through B complex elements, including folic acid, the patients suffering from Hyperhomocysteinemia have little folic acid because the relationship is inverse between homocysteine and folic acid in T2DM, folic acid is one of the factors that help enzymes convert homocysteine into methionine or cysteine. [20].

One study found the relationship between type 2 diabetes and single or combination vitamins. Supplementation has been shown to improve metabolism in patients with diabetes. Folic acid absorption is significantly reduced by prolonged use of metformin, which is the number one drug needed in trouble-free diabetes, accordingly this nutrient has been determined poor in the sickness and most possibly want to be supplemented regularly. Sufferers the usage of metformin in the course of prolonged intervals might also want folic acid [21]. Over time, excessive blood sugar tiers damage millions of nephrons -tiny filtering gadgets inner each kidney. So, it is seen diabetes is one of the necessary factors can be a motive of renal mortality and morbidity and diabetic nephropathy can be lead to renal failure, this is going on if the blood sugar non-controlling [22]. Urea is the end product of protein breakdown that shaped by means of the liver, and it is regarded as an vital indicator for Kidney characteristic. Either the increased concentration of urea is due to a weakness in its composition as a final result of impaired kidney or liver function or due to impaired protein metabolism. Creatinine is the end product that is completely filtered out in the blood and excreted with urine. In diabetics, awareness of the results of creatinine is associated with impaired kidney function. [23]. Not every diabetic patient develops diabetic nephropathy, and the onset of diabetic nephropathy can be prolonged through various therapeutic interventions [24]. And our study agreed with other studies where it was reached Control of blood sugar is an essential condition for the prevention of renal insufficiency and thus maintain normal levels of urea and creatinine in the blood, this may be due to the fact that the patients are not suffering from problems related to renal filtration and did not suffer from diabetic nephropathy (DN) [25]. most of the patients who were sampled upon In our study follow a diet, do not smoke, are satisfied with taking their treatments, and do not suffer from problems with kidney filtration

or from diabetic nephropathy, and that treatments are available and cheap, and tests are conducted from 3 to 6 months. So, these patients maintained acceptable levels of blood sugar and thus didn't affect the function of the Kidney, thus the levels of urea and creatinine didn't rise.

Conclusion

This study demonstrated the strong negative Correlation between IL-37 and Folic acid and the important role for them in predicting and monitoring disease and possibly in the treatment of type 2 diabetes patients.

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