ORIGINAL ARTICLE

CONTENTS

Evaluation of serum levels of calprotectin, lactoferrin and zinc in patients with type II diabetes mellitus

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ABSTRACT

Aim: The current study aimed to evaluate the role of serum calprotectin, lactoferrin and serum zinc in patients with type II diabetes mellitus.

Materials and Methods: Sixty subjects have been investigated in this study, (40) patients with T2DM and 20 apparently healthy participants (control group) during the period between October 2021 to January 2022. Zinc was measured using a calorimetric method, while calprotectin and lactoferrin were measured by ELISA.

Results: The findings also revealed that levels of serum calprotectin and lactoferrin have significantly increased in in patients with T2DM as compared to healthy subjects. The result also showed that serum zin is decreased in patients groups. The correlation matrix exhibited that there was a strong positive correlation between calprotectin and lactoferrin, a significant negative correlation between zinc and calprotectin.

Conclusions: It was concluded that high serum calprotectin and lactoferrin indicated a strong inflammatory status in T2DM patients. Zinc is likely to be negative affected by the high inflammatory response indicated by that high serum calprotectin.

KEY WORDS: calprotectin, lactoferrin, zinc, T2DM, inflammation

Wiad Lek. 2025;78(2):288-294. doi: 10.36740/WLek/201198 DOI

INTRODUCTION

Diabetes mellitus still remainsone of the most worldwide concerning health problems in the current time, with high prevalence and spread in developing states, it is expected that at the time of 2030, the incidence of DM in developing countries tend to increase by 170%, while developed countries may show only 42% increase in DM incidence [1]. Diabetes mellitus is considered as a chronic hormonal disorder, it develops when insulin cannot be secreted sufficiently by β-cells in the pancreas, or/and if the cells cannot effectively utilize insulin [2]. Calprotectin has been classified as an internal activator of the membrane receptors spanning and expressing on immune cells like dendritic cells and macrophages. Therefore, calprotectin is suggested to perform as an endogenous differentiational biomarker for phagocytic cells and as an exterior protein complex, like that's called as a damage-associated molecular pattern (DAMP) [3]. Increased concentrations of serum calprotectin have been observed to be a predicter for vascular changes in patients with type 2 diabetic (T2DM). There was a positive significant correlation has been reported between HbA1c in patients with T2DM and calprotectin levels. This finding suggested that blood glucose levels or glucose metabolites may have effect on calprotectin metabolism of in diabetics [3]. Lactoferrin (Lf) has been known to be found in high levels in human and mammalian milk, in addition to be present in small quantities in exocrine fluids (i.e., salivary secretions, semen, tears, gastrointestinal secretions, vaginal secretions) and inside body cells (i.e., white blood cells, enterocytes, adipocytes, and neutrophils). Lactoferrin is an iron-binding glyco-protein that has antibacterial activity; it also enhances immunological defense mechanisms against invading microorganisms [4]. Thus, it was found that lactoferrin reduces oxidative stress, inflammatory response and apoptotic processes, which are the main mechanisms involved in the development of various cardiac metabolic disorders [5, 6]. Zinc is considered as an essential element for synthesizing insulin into hexamic structure that is stable structurally and functionally. Zinc is also associated to

Table 1. Demographic characteristics of patients and control groups

Indicators		Patients No. = 40		Control No. = 20		Chi Square	P value (Sig.)
		Freq.	Percent	Freq.	Percent		
Age/Years	20-24	13	32.5	7	35.0	0.05	0.97(NS)
	25-29	10	25.0	5	25.0		
	30-34	17	42.5	8	40.0		
Gender	Male	23	57.5	8	40.0	1.64	0.20(NS)
	Female	17	42.5	12	60.0		

NS: Non-significant at P value >0.05.

Table 2. Differences in calprotectin and lactoferrin between patients and healthy groups

Indicators	Patients No. = 40		Control No. = 20		Independent t-test	P value (Sig.)
	Mean	SD	Mean	SD		
Calprotectin (µg/dl)	70.41	40.69	36.28	7.73	5.12	0.000 (HS)
Lactoferrin (µg/dl)	8.77	4.55	6.41	1.29	2.33	0.02 (S)

SD: Standard Deviation; HS: High Significant at P value <0.01; S: Significant at P value <0.05.

Table 3. Pearson correlation coefficient (r) between calprotectin, lactoferrin and zinc in patients with T2DM

Markers	Serum Zinc	Calprotectin
Calprotectin	- 0.509**	
Lactoferrin	- 0.101	0.668**

** High Significant at P 0<0.01

the synthesizing, storing and secreting insulin. In addition, zinc has a protective effect on pancreatic tissue from oxidative stress as a cofactor for the enzyme of superoxide dismutase [7]. In hypozincemic status, the synthesis, storage, and action of insulin can be altered. It has been observed that decreased levels of zinc in T2DM resulted from excretion of zinc through urine or may be because of zinc loss from body cells when glucose is transported into muscles [8].

AIM

The goal of the current study was to evaluate the role of serum calprotectin; lactoferrin and serum zinc in patients with type II diabetes mellitus.

MATERIALS AND METHODS

Sixty subjects have been investigated in this study, 40 patients with T2DM and 20 apparently healthy participants (control group). The study was conducted at the Endocrine Center in Al-Sadr Medical City in Al-Najaf province, in Iraq, during the period between September 2021 and January 2022. Blood sampling (10 ml) had been done after 12 hours of fasting. After separation of serum, and the concentration of zinc was measured by a calorimetric method and spectrophotometry. Calprotectin and lactoferrin were measured by ELISA kits.

Statistical analysis was conducted by SPSS program (version 25) which included descriptive statistics (percentage and frequency) and inferential statistics (t-test and Chi-square test). Pearson Correlation Coefficient (r) was utilized to assess correlation between quantitative variables.

RESULTS

The demographic indicators of both patients and control groups reveal no notable discrepancies in terms of age or gender distribution. The age categories (20-24, 25-29, and 30-34 years) are evenly represented in both groups, with a chi-square test indicating a P-value of 0.97, which is deemed non-significant. Additionally, there is no significant difference in gender distribution; males make up 57.5% and 40% of the patient and control groups, respectively, while females represent 42.5% and 60%, accompanied by a P-value of 0.20 (Table 1). Serum levels of calprotectin and lactoferrin have been evaluated in patients and control groups. The results exhibited a significant increase P<0.05 in calprotectin (µg/dl) in patients compared to healthy group (Table 2 and Fig.1). The same table revealed that there was significant increase P<0.05 in lactoferrin (µg/dl) in patients compared to control group (Fig.2). Regarding serum zinc, a comparison of serum zinc levels between control groups and patients was presented, revealing

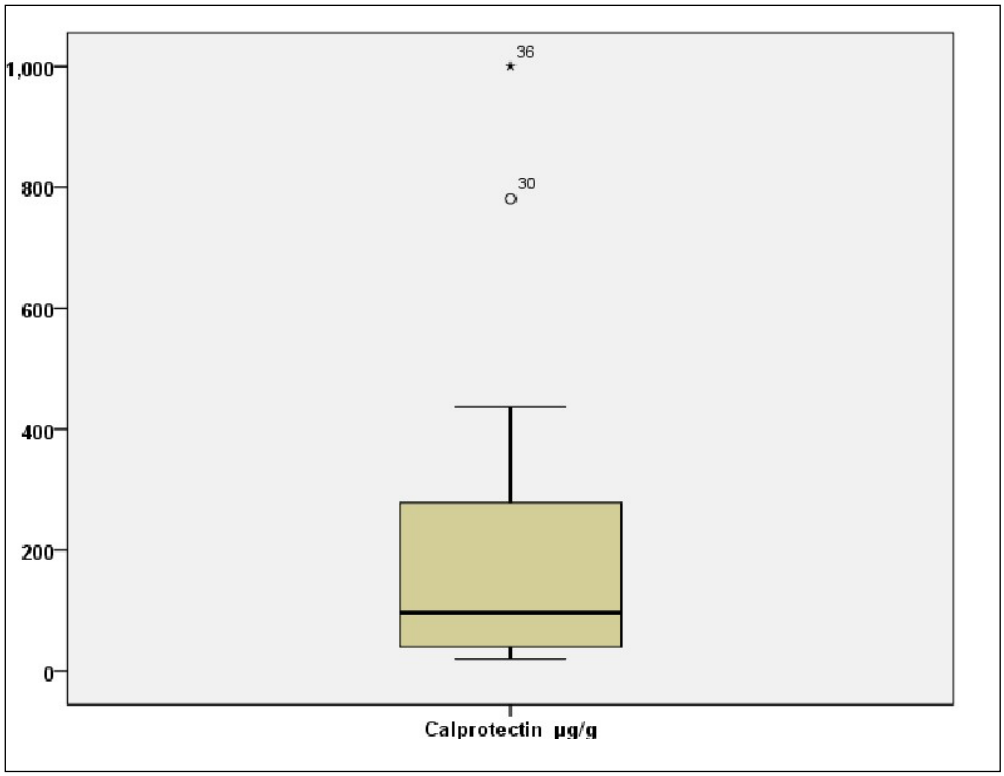

Fig. 1. Distribution of calprotectin in patients with T2DM.

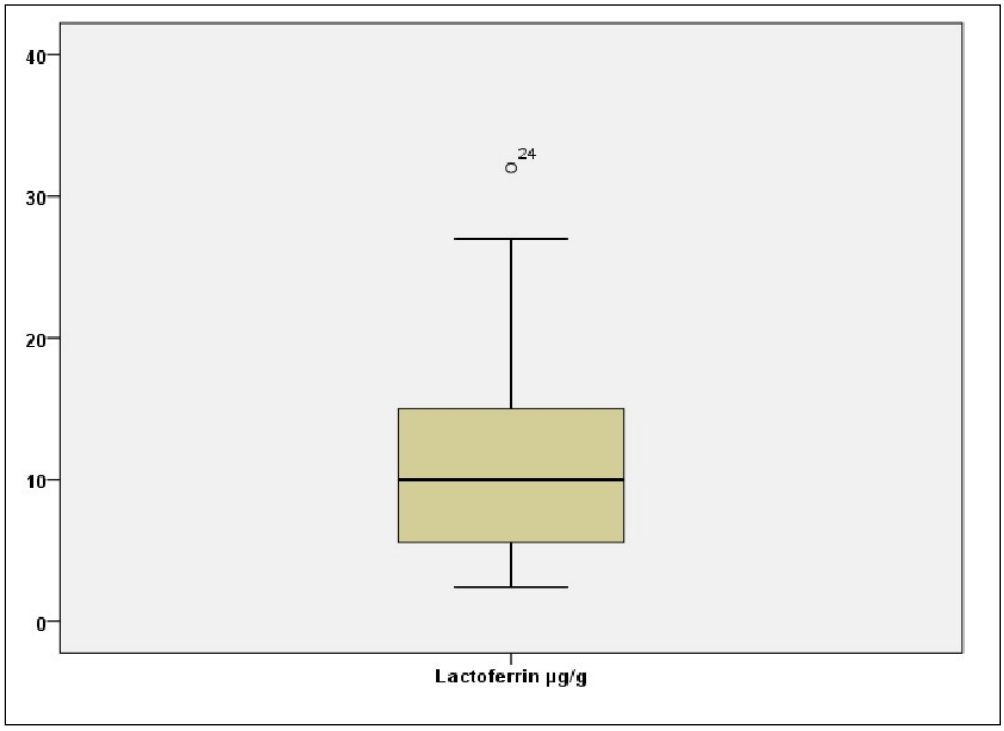

Fig. 2. Distribution of lactoferrin in patients with T2DM.

a significant difference (P-value < 0.001). In the patient group, the average serum zinc level is 61.95 µg/dl, which is considerably lower than the control group's mean of 82.4 µg/dl. The error bars demonstrate the variability within each group, indicating a more restricted range for patients in contrast to the controls (Fig.3, Fig.4).

The correlation test has been achieved by Pearson correlation coefficient (r) after assessing the normality of data (table 3). There was a negative high significant correlation P<0.01 between serum zinc and calprotectin r = -0.509; the findings also pointed out that there is a positive significant correlation P<0.01 between serum Lactoferrin and calprotectin r=0.668.

DISCUSSION

Calprotectin consists of two proteins subunits bounded by calcium atom (S100A8 & S100A9), from which the

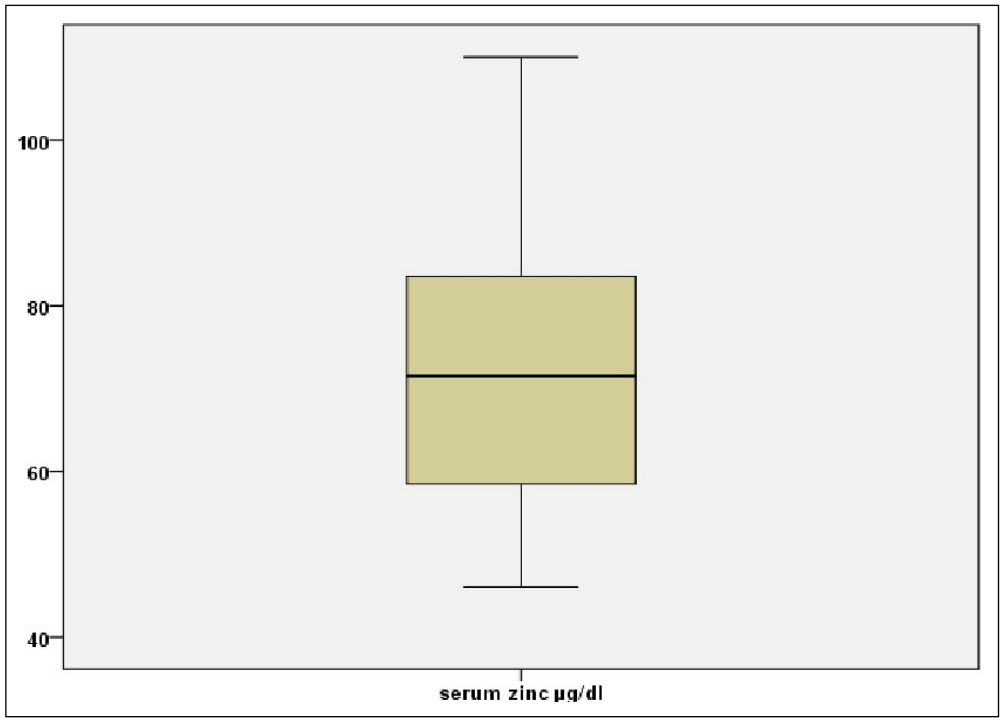

Fig. 3. Distribution of zinc levels in patients with T2DM.

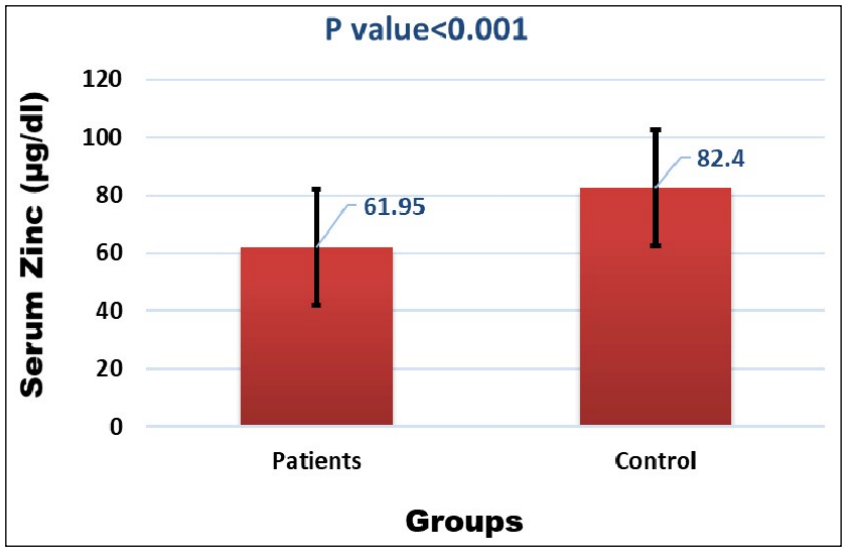

Fig. 4. Differences in zinc between patients and control groups.

name of calprotectin is derived. Calprotectin is classified a strong proinflammatory marker that was observed to increase in chronic inflammation like irritable bowel syndrome (IBS), atherosclerotic lesions, different types of arthritis, and immunological rejection [4]. The present study has found that calprotectin in increased in diabetic patients, this result is supported by previously published studies conducted by Pedersen et al. [9] and Zhang et al. [10]; they found that serum calprotectin exhibited a significant increase in type II diabetic patients. Calprotectin is reported to be secreted by neutrophilic and monocytic cells, which are increased during inflammatory responses. Diabetic patients are basically considered to be under chronic inflammation conditions. Ortega et al. and Mortensen et al., found that plasma concentrations of calprotectin were correlated with inflammation independently of obesity status in T2DM patients [11, 12]. Pedersen et al. [9] observed that T2DM patients tended to have increased levels of serum calprotectin, which were related to myocardial ischemia. Calprotectin was found to be effectively released from macrophages in response to their phagocytic activity and was found to be correlated with inflammatory response more than two decades. It was also found that glucose or/and metabolic end of glycation may have effects on calprotectin metabolism especially high levels in diabetic patients [4]. Regarding Lactoferrin, the findings of the present study agreed with Mohamed & Schaalan [5], they found that lactoferrin was significantly increased in patients with diabetes compared to control group.

Lactoferrin is a transferrin-family iron-binding glycoprotein with a wide range of immunological and biological functions, including antibacterial, immunomodulatory, and anticancer capabilities. Despite being a member of the transferrin family, most of these functions are not expected to be linked to its capacity to bind iron [13]. It has been reported that high lactoferrin levels 931±387 ng/mL are found in moderately obese patients with T2DA more than those of severely obese, nondiabetic subjects. Serum lactoferrin concentrations are greatly associated with insulin resistance (IR) despite lipid profile levels. lactoferrin was reported to be higher in older T2D patients, indicating that unsuccessful therapy may be related to lactoferrin high levels, as well as, high levels of baseline lactoferrin provide a strong prediction among patients with newly diagnosed diabetes for the long-term risk of deadly ischemic heart disease [14]. Some previous studies had suggested ant-diabetic effects for lactoferrin; Mohamed, & Schaalan first suggested that the anti-inflammatory, hypolipidemic and hypoglycemic effects of IF were controlled through the TLR-4, NF-B, SIRT-1 axis, a necessary pathway for signaling that activates anti-inflammatory transcription regulatory factors, in addition to suppression of TNF- and IL-1, in a obese children cohort of T2DM. In a colitis experiment, proinflammatory cytokines have been inhibited by lactoferrin which reveals that lactoferrin, secreted at the inflammation site by secondary granules of active neutrophils, may permit an inhibitory feedback pathway to avoid increased neutrophil aggregates and stimulation [5]. Vengen et al. [14] reported that an increased lactoferrin levels at baseline in newly diagnosed diabetic patients may reflect a more active proinflammatory state and, as a result, a higher risk of cardiovascular disease. Lactoferrin could also be used as a predictive factor for neutrophil count, which is linked to inflammation levels. The neutrophil count is a well-studied predictor of coronary disorders. Lactoferrin is a protein found in neutrophil granules that aid in the progression of an inflammatory response. The present study has shown that there is a significant decrease ($P<0.05$) in serum zinc level in diabetic patients in comparison to healthy group as illustrated in table 3. These results come in agreement with Farooq et al. [15], they found that patients with T2DM have Zn deficiency compared to normal subjects. However, Rusu et al. [16] revealed that the plasma levels of zinc in patients with diabetes were either normal or higher than that of healthy individuals; they attributed that to the occurrence of vascular complications as a trigger for higher serum zinc level in patients with diabetes. Zinc plays a significant task in how muscle and adipose cells use glucose. It serves as a cofactor which activates enzymes inside the cells that took part in glucose, lipid and protein metabolism. Diabetes impacts zinc homeostasis in a variety of ways, though the declines in total body zinc are most likely due to hyperglycemia rather than any main diabetes-related lesion [8]. It was proposed that low Zn levels seen in the diabetic community resulted from the reduced gastrointestinal absorption and high rate of urinary excretion [15]. Previous studies revealed that high blood glucose may have impact on the active transport of Zn secretion into the renal tubular resulting in more zinc to be excreted in urine. In addition, Zn tends to elevate insulin sensitivity by enhancing the insulin molecules binding ability to their receptors [8]. It was also reported that decreased levels of zinc in patients with diabetes are due to loss of zinc molecules as they enhance glucose translocation into muscles [7]. Recently, it was found that mean HbA1C concentration in newly diagnosed T2DM patients exhibited an inverse association with serum zinc levels with Pearson correlation coefficient about $r=-0.804$ indication a strong negative correlation between these two biomarkers [15]. Another study conducted by Seo and his co-workers and found that serum Zn concentration in men was inversely correlated with high fasting blood glucose and shown to have a positive correlation with high TGs, while a negative correlation was seen between serum Zn and HDL cholesterol levels [17]. There is a still a controversial opinion about which primarily effect on the other, diabetes and hyperglycemia on metabolism of zinc or the changes in homeostasis of zinc effect on glucose metabolism. Saha-Roy and his team suggested that low concentrations of zinc can interfere with the function of pancreas and plays a significant role in the pathogenesis of diabetes mellitus [7].

CONCLUSIONS

The current research reveals substantial changes in serum concentrations of calprotectin, lactoferrin, and zinc in patients when compared to healthy controls. Notably, patients showed significantly increased levels of calprotectin ($P<0.05$) and lactoferrin ($P<0.05$), indicating their possible use as biomarkers for the condition being studied. In contrast, serum zinc levels were markedly lower in patients (mean 61.95 µg/dl) than in controls (mean 82.4 µg/dl), with a highly significant difference ($P<0.001$). Furthermore, a Pearson correlation analysis indicated a strong negative correlation ($r=-0.509$, $P<0.01$) between serum zinc and calprotectin, alongside a positive correlation ($r=0.668$, $P<0.01$) between serum lactoferrin and calprotectin. These results highlight complex interactions among these biomarkers, emphasizing their potential diagnostic and prognostic significance in the condition under examination.

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The authors want to thank the patients and their families for their cooperation during the study period and express their thanks to the staff of the Al-Sadr Medical City, in Najaf City, in Iraq for their support and cooperation during sample collection.

This case-control study was approved by the medical ethics committee in the Faculty of Medicine/Kufa University (Reference №: MEC-16 on June 21, 2020).

CONFLICT OF INTEREST

The Authors declare no conflict of interest

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RECEIVED: 28.11.2024
ACCEPTED: 05.02.2025