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A Review of Lactoferrin Inflammatory Role in Type 2 Diabetes Mellitus with Neutrophil Dysfunction

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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Review Article

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ABSTRACT

Lactoferrin (LF) is a protein that plays important roles in many diseases including diabetes mellitus (DM). DM is one of the most challenging health concerns of the 21st century. At least 30% of the diabetic population is undiagnosed at any one time, so effective and early diagnosis is of critical concern. Several of the body's chemicals, such as enzymes, electrolytes, and proteins, have been used as biomarkers in the diagnosis of diabetic diseases. Detection of LF is considered an important sign of type 2 diabetes (T2DM), due to its activity as an anti-inflammatory agent and in the down-regulation of pro-inflammation. LF is produced by glandular epithelial cells and neutrophils, and a decrease in its concentration is linked with the dysfunction of neutrophils in many diseases. Neutrophils are the first line of defence against pathogens that invade the human body during inflammation. Therefore, the health of neutrophils can be employed as a biomarker in the diagnosis of diseases such as diabetes. A decrease in LF concentrations in T2DM could result in increased levels of inflammatory markers that are associated with the inflammation activity. Increased understanding of the link between LF concentration and development of T2DM should improve early diagnosis and treatment outcomes.

LF is identified through use of various techniques such as immunoassay, proteomics, and spectrometry. The aim of this review is to summarise each pathway and some of the most relevant LF biomarkers that may be used to monitor the development or progression of diabetes and its complications, and the link between levels of LF and neutrophil dysfunction in T2DM. Moreover, the objective of this review is to show the most common LF analysis that may be useful in the clinical diagnosis of T2DM and discuss to what extent this analysis method can be a tool for prognostic and diagnostic work.

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1. INTRODUCTION

Lactoferrin (LF, also known as lactotransferrin) is a protein that is produced and released by glandular epithelial cells and is detected in neutrophil secondary granules. LF is a functional glycoprotein with an estimated molecular weight of 80kDa and 690 amino acid residues [1,2]. It is found at high levels in human and bovine milk, and in smaller amounts in exocrine secretions (such as saliva, tears, sperm, vaginal fluids and gastrointestinal fluids) and cells (i.e. neutrophils, enterocytes and adipocytes) [3]. LF is a member of the glycoprotein family and has multifunctional properties. It plays an important role in the immune defence systems of the vaginal, stomach and ocular mucosa. When inflammatory stimuli are present, LF expression is enhanced in those areas, and this enhancement limits inflammatory cytokine production and the ability of lipopolysaccharide endotoxins to bind to inflammatory cells [4].

During infection, neutrophil secondary granules release increased amounts of LF at inflammatory sites to control the physiological homeostasis state [5]. LF is important in the physiological system and is used as a biomarker for many inflammatory diseases, including type 2 diabetes (T2DM). T2DM, also known as insulin-independent diabetes, is linked to obesity and insulin resistance (IR) in the peripheral tissues [6]. T2DM begins to develop several years before it is diagnosed; according to the global guideline of the International Diabetes Federation, between 30% and 90% of T2DM patients are undiagnosed at any one time [7,8]. Improved understanding of the mechanism of action of T2DM will aid in the exploration of the marker that can lead to early diagnosis. The origin of the increased inflammatory activity in T2DM is virtually unknown, yet the first evidence of a connection between inflammation and diabetes was uncovered more than 100 years ago [9,10].

Biomarkers can be used to help researchers to better grasp the origins of illness. Biological indicators include proteins, genetic and metabolic markers. Inflammatory biomarkers such as orosomucoid, tumour necrosis factor-α (TNF-α), transforming growth factor-β, vascular endothelial growth factor and monocyte chemoattractant protein-1, as well as oxidative stress markers such as 8-hydroxy-2-deoxyguanosine, may be useful for the diagnosis or monitoring of diabetic complications. Biomarkers can also be employed in biological systems to identify, characterise and observe the expression of proteins. Protein biomarkers are extremely useful to predict long-term mortality in diabetic patients. New biomarkers can be found in tissues and/or biofluids (blood, serum, plasma and urine) [11]. Protein biomarkers have been identified in biofluids, tissues and cells, particularly in T2DM patients [12]. Apolipoproteins, such as apolipoprotein A1, the major component of plasma-bound high-density lipoproteins (HDLs), have been found useful as protein biomarkers [13].

Concentrations of LF *in vivo* vary according to the type and severity of the disease. Various analytical methods are available to measure these concentrations in order to screen for the presence of diseases such as T2DM, to evaluate their severity, to monitor their progress and to offer prognoses.Neutrophils are polymorphonuclear entities (PMNs) and phagocytic leukocytes that form the first line of defence against invading pathogens in the human body. During inflammation that is induced by tissue injury, they are also essential effector cells [14]. PMNs have roles in chemotaxis, attachment to the endothelium and foreign agents, phagocytosis and microbicidal activity. PMNs have the capacity to enter diseased tissues and destroy invading bacteria by producing a variety of harmful chemicals such as ROS, proteases and LF [15]. The Neutrophils are dysfunctional due to infection agents [16].

The aim of this review is to describe the potential of use of LF as a diagnostic biomarker for T2DM with neutrophil dysfunction and to consider several clinical chemistry analytical techniques that can be used to detect the level of LF in various biological samples. It also highlights the challenges involved.

2. MECHANISMS OF TYPE 2 DIABETES MELLITUS

T2DM is the world's most prevalent and clinically significant metabolic disease. It has become a global epidemic and a huge healthcare burden in recent decades as the number of people with T2DM has increased. In 2013, there were an estimated 382 million T2DM patients worldwide [17], and by 2035, this figure is expected to increase to more than 590 million [18,19].

Diabetes is a "metabolic disease of many etiologies defined by persistent hyperglycemia with disturbances in carbohydrate, lipid, and protein metabolism arising from abnormalities in insulin production, insulin action, or both", according to the World Health Organization [20]. Development of T2DM is closely linked with hereditary variables such as decreased levels of insulin secretion and IR, as well as environmental factors such as obesity, lack of exercise, overeating, stress, inadequate calorie consumption, alcohol use, smoking and ageing [21].

Insulin is produced by β-cells, which first generate pre-proinsulin. During the maturation process, pre-proinsulin undergoes a structural change with the help of many proteins, culminating in production of proinsulin. After that, proinsulin is degraded into C-peptide and insulin. Insulin is retained in granules throughout maturation until insulin release is activated. The release of insulin is largely induced by a reaction to high blood-glucose levels. Other variables, such as levels of amino acids, fatty acids and hormones, can also cause insulin to be released. When blood-glucose levels rise, glucose transporter 2 is used primarily by β-cells to take it in. When glucose enters the cell, it causes the intracellular ratio of adenosine triphosphate to adenosine diphosphate (ATP/ADP) to rise and the ATP-dependent potassium channels in the plasma membrane to close. This process triggers glucose catabolism, which causes the membrane to depolarise and enables Ca^{2+} to enter the cell through voltage-dependent Ca^{2+} channels. Insulin exocytosis is triggered by an increase in intracellular Ca^{2+} concentration, which causes the priming and fusing of secretory insulin-containing granules to the plasma membrane [22,23] (Fig. 1A).

According to recent research, β-cell dysfunction in T2DM may be mediated by a complicated network of interactions between the environment and numerous biochemical processes that occur in the cell. Excessive eating, as with obesity, is associated with hyperglycaemia and hyperlipidaemia, which promote IR and chronic inflammation [24]. The β-cells are subjected to toxic factors such as inflammation, inflammatory stress, metabolic/oxidative stress and amyloid stress under these conditions. These toxic factors can cause loss of islet integrity owing to genetic susceptibility differences [25,26]. Excess amounts of free fatty acids (FFAs) and hyperglycaemia stimulate the apoptotic unfolded protein response pathways, resulting in β-cell malfunction. Obesity-related lipotoxicity, glucotoxicity and glucolipotoxicity induce metabolic and oxidative stress, which lead to β-cell death [26,27]. Furthermore, prolonged high levels of glucose enhance proinsulin biosynthesis and the development of islet amyloid polypeptides in β-cells, as well as an increase in production of reactive oxygen species (ROS) [27] (Fig.1B).

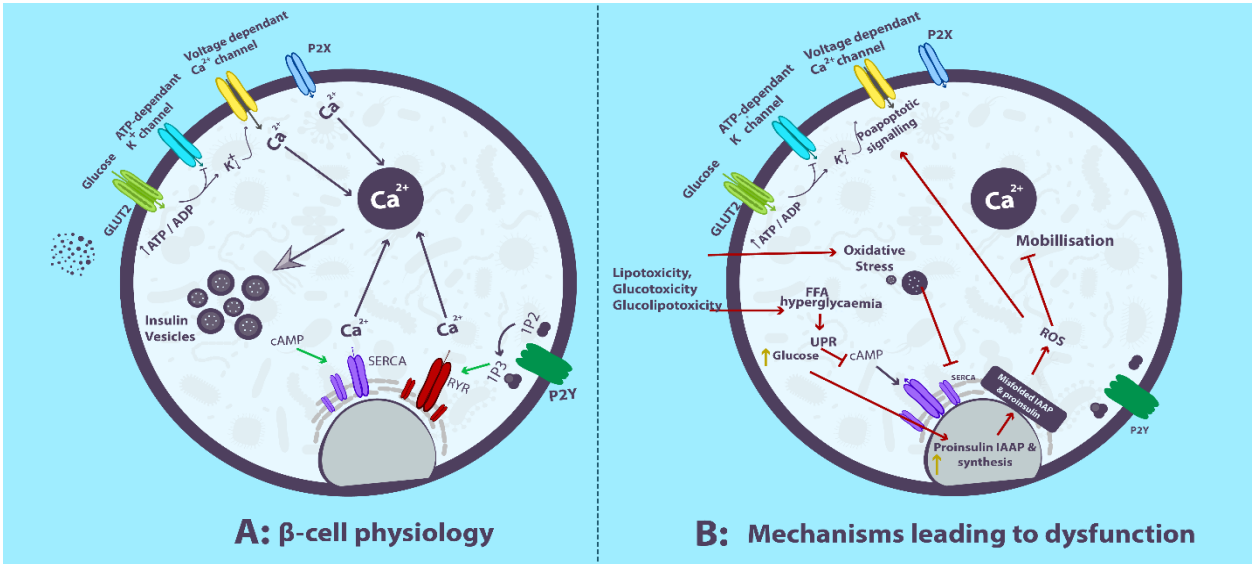

Fig. 1. β-cells in healthy circumstances (A) and during dysfunctional processes (B). Diagram adapted from Galicia-Garcia et al. [28]

3. THE ROLE OF INFLAMMATION IN TYPE 2 DIABETES MELLITUS

The first evidence of a connection between inflammation and diabetes was discovered over a century ago. The role of inflammatory processes in the development and progression of T2DM has received greater attention than it has in type 1 diabetes due to an important mechanism. Increases in levels of inflammatory markers have been seen in seemingly healthy people who subsequently acquire T2DM. This finding suggests that inflammation starts early in the period of reduced glucose tolerance and prior to T2DM diagnosis [24,29,30].

IR has long been thought to be a key factor in the pathophysiology and progression of T2DM. IR begins prior to the onset of T2DM, when β-cell breakdown and insulin insufficiency result in decreased glucose tolerance. Several elements, including genetics and environmental effects, obesity, and other diseases associated with chronic inflammation or infection, have been related to the development of IR in people with impaired glucose tolerance and T2DM [24].

Fig. 2 shows how inflammation develops in T2DM, as described by Donath and Shoelson [29]. Overeating causes levels of blood glucose and FFAs to rise, and this leads to metabolic stress in various tissues. This stress triggers the production of a variety of pro-inflammatory cytokines and chemokines. Immune cells are drawn in, and this process contributes to tissue inflammation [24,29,30].

IR is linked primarily to a variety of pro-inflammatory and/or oxidative stress mediators, including pro-inflammatory cytokines such as interleukins (ILs) 1β and 6, and TNF-α, as well as a variety of chemokines and adipocytokines [31,32]. These pro-inflammatory cytokines can cause systemic insulin sensitivity and glucose imbalance as they directly increase IR in adipocytes, muscle cells and hepatic cells. Increased levels of these pro-inflammatory cytokines cause the liver to generate and release C-reactive protein, plasminogen activator inhibitor-1, amyloid-A, 1-acid glycoprotein and haptoglobin. These proteins first appear in the early stages of T2DM, and their blood levels grow as the disease progresses [33].

Several studies have revealed the occurrence of various inflammatory responses in β-cells and peripheral tissues. These studies report that IL-β is a master pro-inflammatory mediator that activates a plethora of other pro-inflammatory cytokines and chemokines. Once inflammation is triggered, it has a negative impact on β-cells in pancreatic islets, resulting in decreased insulin production. Similarly, IL-β plays a critical role in the induction of inflammation in peripheral tissues, which contributes to the development of IR in these tissues [34,35].

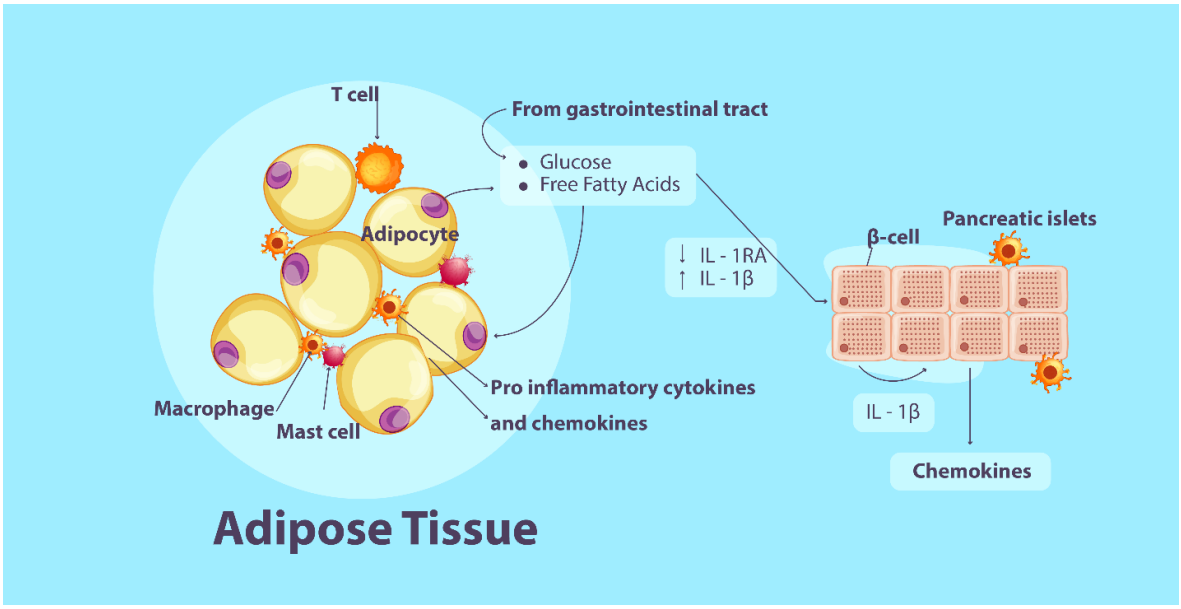

Fig. 2. How inflammation develops in T2DM. Diagram adapted from Donath et al. [29]

TNF-α has been identified as a key cytokine that is implicated in IR. TNF-α has metabolic effects in peripheral tissue as it alters the expression of genes involved in lipolysis and lipogenesis, which results in a rise in FFA concentrations. Higher levels of hepatic gluconeogenesis and IR in skeletal muscle are linked with increased FFA levels. This increase in FFAs is also linked with insulin hypersecretion, which can lead to a reduction in insulin secretion capacity [36]. TNF-α plays an essential role in insulin signalling system as it inhibits activity of the insulin receptor tyrosine kinase in adipocytes, which leads to decreased phosphorylation and activation of insulin receptor substrate-1 and so limits cell responsiveness to insulin. TNF-α has also been linked to a reduction in the expression of genes that encode proteins that produce insulin receptor substrates [37].

Ageing is linked to higher amounts of IL-6 in the blood, which can be linked with increased IR [38]. The process by which IL-6 triggers IR is complex and multifaceted. It not only stops non-oxidative glucose from being metabolised, but it also inhibits the activity of lipoprotein lipase, which raises triglyceride levels in the blood [39]. Furthermore, the presence of IL-6 activates the cytokine signalling suppressor, which may inhibit the activation of cytokine-mediated insulin receptor transcriptional factors [40]. As a result, IL-6 is considered a critical biomarker for IR development.

4. NEUTROPHILS AS BIOMARKERS IN DM

Interactions with the vascular endothelium regulate neutrophil migration from the circulation to the site of inflammation. Primed neutrophils actively manufacture and release cytokines, chemokines, leucotrienes and prostaglandins, and present antigens, by virtue of their vast numbers inside inflammatory tissue, which leads to local generation of inflammatory mediators. In response to a variety of stimuli, including TNF-α, neutrophils have been demonstrated to manufacture and release IL-8 [41,42]. Activated neutrophils have also been shown to produce IL-1, IL-6, IL-12, TNF-α and oncostatin M, all of which can stimulate the activity of neutrophils and other immune-system cells [43]. It is important to find out where various neutrophil phenotypes are made during severe inflammation events. Neutrophil cell-surface markers or their derivatives can be employed as biomarkers in disease diagnosis. Chronic inflammation is a feature of T2DM, which involves humoral components as well as several kinds of white blood cells, such as mononuclear and PMN leukocytes. The development of T2DM has been linked to an increase in neutrophil count and phagocytic dysfunction. This is related to the well-known theory that oxidative stress, which is generally created by neutrophil activity, causes diabetes problems [44].

T2DM is now recognised as an inflammatory condition that is associated with IR and abnormal endothelial vascular reactivity. Insulin has been shown to have a substantial regulatory influence on the functional activities of immune cells [45,46]. Insulin's priming effect on PMN activity can be viewed as the body creating a wide defence to support the major immunological response to antigen exposure, and this response is aided by meal intake [47]. Insulin sensitivity declines with age, and this situation adds to the immune system's age-related deterioration, particularly after meal consumption [46].

PMN function is influenced by the conditions caused by T2DM, age-related IR, diet and lifestyle. In human and animal models of diabetes, abnormalities in neutrophil adhesion, chemotaxis, phagocytosis, ROS generation and microbicidal activity have been reported [48,49]. The occurrence of hyperglycaemia reduces the activity of glucose-6-phosphate dehydrogenase (G6PD) and glutaminase enzymes, while it increases the activity of phosphofructokinase [48]. Reduced G6PD activity impairs the development of the pentose-phosphate pathway as well as neutrophil activities [50].Even when the subject’s glycaemic index is incorrect, insulin enhances neutrophil phagocytosis and ROS generation. This finding suggests that insulin has a direct effect on neutrophils [48]. Furthermore, increased levels of circulating FFA and triacylglycerol promote IR as well as neutrophilic inflammation [51,52].

Changes in immune-cell activity may explain why the T2DM and older populations suffer infections more frequently than other people. Research has demonstrated that treatment of hyperglycaemia with insulin can lead to restoration of diabetic patients' impaired PMN functioning. Glucose intake and glycogen metabolism in PMNs are both insulin-dependent, despite the fact that PMNs do not require insulin in order to absorb glucose. Following insulin therapy, insulin receptor expression has been found to be linked to PMN chemotaxis in both young and old individuals. In insulin-resistant and elderly

people, antimicrobial protein synthesis in PMNs is altered, and it is reduced in all humans after intravenous endotoxin injections under hyperglycaemic conditions [15]. Elgazar-Carmon et al. (2008) discovered that a high-fat diet caused significant neutrophil recruitment to intra-abdominal adipocyte tissue; this recruitment peaked after three to seven days and subsequently faded. The researchers theorised that neutrophil recruitment was necessary to kickstart the inflammatory response to high-fat meals. These neutrophils may produce chemotactic factors, which enable macrophage infiltration and the continuation of an inflammatory state in adipose tissue. This chronic inflammatory infiltration is preceded by a short, acute infiltration of inflammatory molecules that are dominated by neutrophils, according to a well-established paradigm in systemic inflammatory processes [53].

5. NEUTROPHIL DYSFUNCTION IN TYPE 2 DIABETES: SPECIFIC MARKERS

In patients with IR or T2DM, levels of antibacterial neutrophil proteins such as LF, bactericidal/increasing permeability protein (BPI) and α-defensins are decreased. The decreased antibacterial ability of neutrophils that occurs in T2DM patients has been found to correspond with the circulating levels of these proteins [54].

BPI is a 55kDa cationic protein that is found in the azurophilic granules of neutrophils. Plasma BPI concentration has been found to be negatively related to metabolic indices and directly correlated with insulin sensitivity and levels of HDLs [55,56].

Human α-defensins are peptides that contain 29-35 amino acids and have high arginine content. In seemingly healthy Caucasian males, significant positive correlations have been found between concentrations of plasma α-defensin, insulin sensitivity, nonatherogenic lipid profile and vascular function [57,58].

Furthermore, numerous investigations have shown that development of T2DM is linked to a change in neutrophil functioning (lower bactericidal ability and higher neutrophil count) [59-61].

6. LACTOFERRIN AS A DIAGNOSTIC MARKER FOR TYPE 2 DIABETES DISEASES

LF levels in the body are elevated during development of an infection or an inflammatory disease, which means that LF can be used as a biomarker for inflammatory disorders. The presence of LF also reduces inflammation as it interacts with macrophages and limits the production of inflammatory cytokines by cells in a similar way to other anti-inflammatory cytokines, according to several studies [62]. Scientists have made many attempts to improve their comprehension of the role of LF in the maintenance of human health [63].

Videm et al. (2007) reported that when a person who displayed traditional risk factors for coronary artery disease was infected with *Chlamydia. pneumoniae*, coronary artery disease would develop only if monocytes/macrophages and neutrophils were activated. According to this study, increased concentrations of LF but not of myeloperoxidase are linked with the occurrence of severe coronary artery stenosis [64]. Furthermore, both at baseline and after a fat overload, circulating concentrations of LF have been reported to be negatively correlated in extremely obese individuals with postprandial lipaemia and production of oxidative stress markers (e.g., catalase and glutathione peroxidase) and C-reactive protein [65].

According to recent studies, LF can be utilised as a biomarker in the detection of inflammatory bowel disease (IBD) [66], Alzheimer's disease (AD) [67] and dry-eye disease (DED) [68]. Clinical grading systems and endoscopy have traditionally been used to diagnose IBD. However, both these methods are costly and show limited accuracy. Previous research has suggested that faecal levels of LF might be useful biomarkers to predict the development of IBDs [69]. As PMN neutrophils degranulate during intestinal inflammation, secondary granules are produced. LF is a key component of secondary granules, and therefore LF concentration increases in cases of IBD. In cases of Crohn's disease (CD) and ulcerative colitis (UC) in children, the levels of faecal LF have been reported to be greater than those in control participants (7.3g/g), although the diagnostic efficacy of this protein in UC patients is reported to be better than in CD patients [70].

It is difficult to diagnose AD early in its development. Current tactics involve combining the techniques of positron emission tomography and magnetic resonance imaging to assess the levels of tau and amyloid proteins in the cerebrospinal fluid [71]. There have been attempts to create a rapid and cost-effective

diagnostic method. Research in AD pathophysiology suggests that bacterial and viral infections may induce onset of the disease and lead to a weakened innate immune system [72]. Saliva is considered the body's first line of defence against infection because it contains numerous antimicrobial proteins. Some reports have linked oral infections to the development of AD [73]. LF is an essential defence component of saliva due to its particular antibacterial properties. Therefore, the measurement of salivary LF levels may offer a diagnostic method for the early detection of AD. González- Sánchez *et al.* (2020) measured levels of salivary LF in order to diagnose prodromal AD and to study the relationship between salivary LF levels and cerebral amyloid-β. The results showed that salivary LF levels did not decrease in other dementias, such as frontotemporal dementia, and that reduced levels of LF could be attributed to the disruption of hypothalamic function due to early hypothalamic amyloid-β accumulation [67].

DED, a common ocular surface disease of multifactorial aetiology, causes many symptoms and visual impairment, sometimes with ocular surface damage [74]. DED is currently diagnosed through use of several tests such as evaluation of the tear osmolarity, the Schirmer tear test and the phenol red thread test [75]. However, these methods are of low accuracy and can be easily affected by environmental factors. The presence of LF in the tear film plays a key role in the avoidance of ocular diseases because of its unique antimicrobial and anti-inflammatory activities [76]. Some recent research has confirmed that the concentrations of LF in tears are significantly different between patients with DED and controls [67,77].

A 1995 study discovered that mesencephalon samples that had been obtained by autopsy from eight patients with histologically confirmed Parkinson's disease (PD) showed a higher content of the LF receptor than samples taken from 13 people with no known history of psychiatric or neurological disorders. This finding kicked off research into a link between levels of LF and the pathogenesis of PD [78]. Additional examination of the mesencephalon cellular distribution revealed significant levels of LF in a wide population of neurons in the substantia nigra (SN) of control individuals. In comparison with control instances, individuals with PD exhibited greater LF levels in the surviving neurons of the SN, according to quantitative analyses. The researchers concluded: "Further research will be required to understand whether LF serves as an iron scavenger and may represent a protective factor, or whether it promotes excessive iron buildup leading to oxidative injury in susceptible neurons" [79]. Two recent investigations separately showed that LF might be useful as a non-invasive PD marker, after they discovered that the levels of LF in the saliva and tears of PD patients were higher than those in the same biofluids of the control group [80,81]. Salivary and lacrimal LF levels could be acceptable as PD markers since they are simpler to collect than blood samples and, more importantly, the amounts of LF in both exocrine secretions are substantially larger than the levels of oligomeric alpha-synuclein. This compound is widely utilised as a marker despite its high prevalence in red blood cells, low concentration in biological fluids and contradictory meta-analysis results, which limit its utility in PD diagnosis [82].

Several studies have shown that LF regulates the production of inflammatory cytokines in a similar way to other anti-inflammatory cytokines. LF has been found to reduce β-cell damage by decreasing production of TNF-α, IL-1β, IL-6 and IL-8 in human mononuclear cells (*in vitro*) while it increases IL-10 and IL-4 production (*in vivo*) [83].

7. CORRELATION OF LF ANALYSIS WITH INSULIN RESISTANCE AND TYPE 2 DIABETES STUDIES

The observation that there was a negative connection between concentrations of circulating LF and of fasting glucose [84,85] and that there was a positive correlation between levels of circulating LF and insulin sensitivity [85] sparked research into the role of LF in glucose metabolism changes. It is possible that LF has a direct impact on IR in peripheral organs [86]. Mohamed et al. (2018) discovered that glucose metabolism in diabetic children was changed compared with that in their control counterparts and that the diabetic youngsters showed a two-fold increase in LF levels [87]. The increased levels of LF helped weight loss by improving insulin action and increasing the activity of the fuel-sensing protein [65].

8. NEUTROPHIL DYSFUNCTION AND LF PRODUCTION

In a broad sense, LF is an acute-phase protein that functions as an "alarmin". Alarmins form a

Table 1. Selected studies of quantitative measurements of LF in T2DM patients

Study	Sample type	Analytical method	Conclusions	Reference
LF concentrations in tears and tear secretion rates were measured in normal and diabetic individuals.	Tears	ELISA	There was no link between the amount of LF in the tears of the diabetic subjects and the length of time they had been diabetic, and there was no variation in the amount of LF in normal tears vs. diabetic tears.	[97]
Diabetic and diabetic with retinopathy patients randomly selected for study of tears.	Tears	SDS-PAGE	Tear film was decreased more in diabetic subjects than in normal subjects.	[98]
In hamsters in which diabetes was induced with streptozotocin, the levels of salivary antibacterial agents such as lysozyme, lactoperoxidase and LF were measured.	Saliva	Gel electro-phoresis	Ratio of LF to total protein in the hamsters was about twice that of the control hamsters. Insulin therapy restored 73% and 74% of the activity of lysozyme and lactoperoxidase, respectively, and the ratio of LF to total salivary protein returned to normal levels.	[99]
The connection between circulating LF, LF gene polymorphisms, dyslipidaemia and vascular reactivity in male humans with glucose intolerance was examined.	Plasma	ELISA	With IR and T2DM, the concentration of circulating LF was reduced. Fasting triglyceride concentration, body-mass index, waist-to-hip ratio, and fasting glucose levels were all shown to be inversely associated with levels of LF, whereas HDL cholesterol concentration was found to be directly related.	[84]
In a Caucasian population, the association between circulating LF and chronic inflammation-associated IR was investigated according to glucose tolerance level.	Plasma	ELISA	LF levels in the blood were found to be substantially lower in patients with impaired glucose tolerance compared with healthy people. It was possible that LF was involved in persistent low-level inflammation and IR.	[85]
The goal of this study was to see how well levels of myeloperoxidase and LF, two neutrophil degranulation products, predicted long-term risk of fatal ischaemic heart disease in patients with T2DM and in healthy people.	Serum	ELISA	There was no significant difference in LF levels between T2DM patients and controls.	[100]
Concentrations of LF were measured in T2DM patients compared with non-diabetic controls.	Serum	ELISA	Levels of LF were greater In T2DM patients than in control participants.	[101]
The concentrations were measured of protective factors in the saliva of individuals with T2DM who had decompensated.	Saliva	ELISA	In decompensated T2DM patients, salivary LF levels were significantly lower than in the control group.	[102]
LF was studied as a biochemical marker in individuals with T2DM and in those with T2DM and peripheral neuropathy (diabetic nerve pain, DNP).	Serum	ELISA	T2DM patients showed significantly higher serum LF levels when compared with the control group, whereas those with DNP showed highly significant increases when compared with both the control and T2DM groups. LF was likewise favourably associated with levels of HbA1c in the T2DM group and negatively with Fe in the DNP group.	[103]

small family of proteins that are produced by neutrophils in response to infection. They play a key role in the modification of immune reactivity in response to a pathogenic encounter or clinical insult [88,89].

LF is expressed and stored in secondary granules by neutrophil leukocytes, which make up more than half of all white blood cells. LF is produced in the blood during neutrophil activation, which occurs at the initial stages of attachment to the activated endothelium. Its concentration can reach 200mg/l (compared with around 1mg/l under normal conditions), especially in inflamed tissues. Microglial cells, which function as resident macrophages in the brain, also produce LF when the brain is inflamed [90]. In a confined area, LF as an alarmin develops conditional connections between neutrophils and dendritic cells [88].

The innate system's armoury that is used to establish microbial balance in mucosal fluids includes LF, released immunoglobulin A and defensins [91]. LF is a multifunctional molecule due to its tendency to interact with microbial and target host-cell surfaces and its high affinity for ferric iron. This affinity deprives bacteria of the free iron they require to flourish [92]. The antibacterial properties of neutrophilic LF, which is generated in high concentration in infected tissues and is probably linked to the chromatin fibril matrix released by neutrophils (neutrophil extracellular traps), are comparable [93].

9. METHODS OF BIOANALYSIS OF LF IN DIABETES PATIENTS

Various bioanalytical techniques have been used to measure levels of LF in biological samples. One of the most popular, due to its specificity and sensitivity, is enzyme-linked immunosorbent assay (ELISA). It is a quantitative, highly accurate, fast technique that can detect molecules in ng/ml concentrations. ELISA can be used to detect the binding of analyte and specific antibodies [94]. ELISA techniques have been reported in many studies of LF levels in DED, Crohn's disease and diabetes [95-97]. Table 1 highlights the evidence that is shown in selected studies and which supports the existence of a relationship between levels of indicators of neutrophil dysfunction (LF) and T2DM. The table also includes references to LF testing techniques. According to the findings shown in the table, T2DM is associated with decreased LF production and/or secretion in neutrophils.

10. CONCLUSION

LF is a protein that plays an important role in inflammation. In many studies, it has been found to play a key role in the development of T2DM. Due to neutrophil dysfunction, LF levels usually affect clinical diagnosis. ELISA techniques have been used widely to detect the concentrations of LF in diabetic patients due to their high sensitivity and selectivity. However, use of other techniques such as LC-Ms/Ms and proteomics should be considered to improve the analysis and understanding of LF activity and quantity in diabetes.

DISCLAIMER

Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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