Role of EBV infection in Type-1 Diabetic nephropathy pathogenesis with related to IL-12 level in patients

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Abstract. Type 1 Diabetic nephropathy (T1DN) is the leading cause of chronic kidney disease globally The primary risk factors for the development of DN are chronic hyperglycemia and excessive blood pressure. In general, microalbuminuria screening should be done yearly, beginning 5 years after diagnosis in type 1 diabetes. This study design to investigate The role of IL-12 in the diagnostic of T1DN, and evaluation the role of IL-12 in patients infected and non-infected with EBVNA IgG. A case-control study design on 70 participates which included 30 patients with T1DN collected from nephrology department in Al-sadder medical city in AL- Najaf, from October 2022 till end of February 2023 and 40 control groups divided into two types ,first 20 apparently healthy and 20 nephropathy patients . Blood sample was collected from all participants to detected IL-12 serum level and EBNA1 IgG by enzyme linked immunosorbent assay (ELISA) . All patients were diagnosis by nephrology specialist. The results showed a that serum IL-12 levels were significantly higher in type 1 diabetic nephropathy patients (27.65 ± 3.78 pg/ml) than in the control groups (12.32± 3.41 pg/ml ; 1.89 ±0.47 pg/ml) respectively at (P= 0.0001).Also The level of IL-12 was significantly elevated in T1DN patients infected with EBNA1 IgG (33.84 ± 4.47) in compare to patients non infected with EBNA1 IgG (13.21 ± 4.36) at P 0.01. Serum levels of IL-12 were significantly increased in T1DN Iraqi patients and increased correlated with infection of EBNA1 IgG.

1 Introduction

Type 1 Diabetic nephropathy (T1DN)**:** is one of the most common microvascular consequences of diabetes mellitus . T1DN is a condition marked by a gradual increase in urine albumin excretion (UAE), high blood pressure, and glomerular lesions that eventually lead to loss of glomerular filtration and, finally, end stage renal failure(ESRF). DN is still the most common chronic kidney disease [1]**.** The mechanisms involved in the development of autoimmune condition are related to a breakage in the central and peripheral tolerance, immune activation of T lymphocytes, and imbalance between Th1 and Th2 inflammatory response with the production of cytokines, leading to the progressive destruction of the β-

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cells and the gradual loss of insulin production[2]**.** Immune and inflammatory responses have been demonstrated to play a significant part in the pathogenesis of DN, although DN has historically not been considered an inflammatory illness. However, new research suggests that renal inflammation plays a significant role in the onset and progression of DN[3] . In type 1DM , macrophages accumulate in the kidneys and become activated, which is linked with prolonged hyperglycemia, glomerular immune complex deposition, and increased chemokine production, eventually leading to renal damage and fibrosis[5]. Cytokines play crucial roles in orchestrating complex multicellular interactions between pancreatic β cells and immune cells in the development of type 1 diabetes (T1D) and are thus potential immunotherapeutic targets for this disorder[6]. Interleukin-12 p40 play important role in T1DM pathogenesis by influences T cell response and T lymphocytes towards the Th1 subset, characterized by production of cytokines leading to cell mediated immunity. In addition higher IL-12 induced autoreactive T cell responses might predispose to self-destructive immunity[7]. Epstein-Barr virus (EBV) is a double-stranded DNA gamma herpes virus consider a constant threat for autoimmunization . EBV could serve as autoimmune initiators for some autoimmune diseases and play a promotional role in majority of the autoimmune diseases[8].

2 Materials and Methods

2.1 Patients and control characterization

A case-control study design on 70 participates which included 30 patients with Type 1 diabetic nephropathy , the age of patients range (8- 47), collected from nephrology department in Al-sader medical city in AL- Najaf, from October 2022 till end of February 2023 and 40 control groups divided into two types ,first 20 apparently healthy with the age range(8-47) and 20 nephropathy patients with the age rang (11- 45)years.

2.2 Sample collection

Four ml was collected from both patients and controls was placed into a gel tube. After the blood had been at room temperature for about 30 minutes to allow for clotting, it was centrifuged for 3 minutes at 4000 rpm, then serum separated and stored at -20 C until used for measuring IL-12/ EBVNA1 IgG according to (Elabscience, USA).

2.3 Statistical analysis

Statistical analysis was carried out by using statistical software (IBM SPSS Statistics 26). the result of IL-12 were expressed as arithmetic mean ± SE. The comparison between infected patients and non-infected patients was analyzed by t-test (two means). p-value (<0.05) was considered significant statistically [9, 10].

2.4 Ethical committee

Ethical approval: The study was conducted in accordance with ethical principles that have their origin in the Declaration of Helsinki. It was performed with patients' consent both verbally and analytically before sampling. The study protocol, subject information, and consent form were reviewed and approved by the local ethics committee according to the document number 397 in 19/1/2023 to get this approval.

3 Results

3.1 Distribution of patients and control subjects according to disease, age and gender

A case-control study design on 70 participates which included 30 patients with Type 1 diabetic nephropathy collected from nephrology department in Al-sader medical city in AL-Najaf. The group of T1DN patients included more male 20 (%) than female 10 (%) **.** In addition to 40 controls group divided into two types ,first 20 apparently healthy with male : female 10:10 (%) for each one and 20 nephropathy patients as shown in table (1). According to means age of patients T1DN group followed by control groups , 23.66±1.04 years versus 25.7 ±3.14 years versus 28.

Table 1. Distribution of study subjects according to type of disease, age, gender, infection and non-infection with virus .

	Autoimmune based glomerulonephritis	**control group**	
	T1DN N= 30	Healthy group N= 20	Nephropathy group N=20
Male	20 (66.6%)	10 (50%)	12 (60%)
Female	10(33.3%)	10 (50%)	8 (40%)
Age Mean ± SE	23.66 ± 1.04	25.7 ± 3.14	28.6 ± 3.15
Age range	- 8- 47	8 - 47	11- 45
< 20	10 (33.3%)	4 (20%)	3 (15%)
20-44	18 (60%)	8(40%)	6 (30%)
>45	2 (66.6%)	6(30%)	8(40%)
infections	21 (70%)	6 (30%)	8(40%)
Non- infections	(30%) 9	14(70%)	12(60%)

3.2 Evaluation serum IL-12 levels in patients with type 1 diabetic nephropathy and control groups

The results showed that serum IL-12 levels were significantly higher in type 1 diabetic nephropathy patients (27.65 ± 3.78 pg/ml) than in the control groups (12.32± 3.41 pg/ml ; 1.89 ±0.47 pg/ml) respectively at (P= 0.0001) . As show in the (Table 2) :

Study group	IL-12	P-value
T1DN (30)	27.65 ± 3.78	0.0001***
Nephropathy groups (20)	12.32± 3.41	
Healthy groups (20)	1.89 ± 0.47	

Table 2. Mean of IL-12 serum level in Type 1 diabetic nephropathy patients compared control groups

3.3 Evaluation of (IL-12) level in type 1 diabetic nephropathy infected patients compared with non- infected in (EBNA1 IgG) :

The level of IL-12 was significantly elevated in T1DN patients infected with EBNA1 IgG (33.84 ± 4.47) in compare to patients non infected with EBNA1 IgG (13.21 ± 4.36) at P 0.01 . As show in figure (1) :

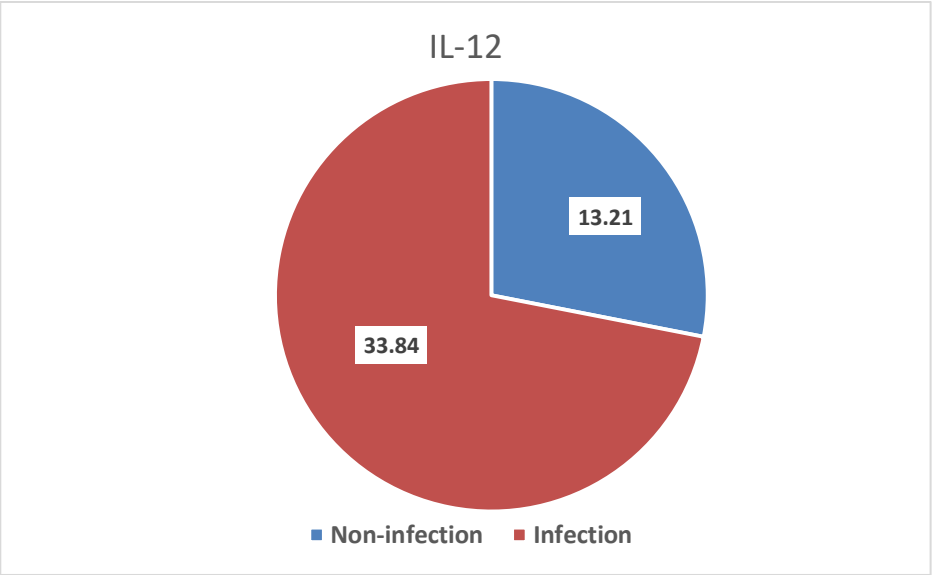

Fig. 1. IL-12 level in type 1 diabetic nephropathy patients infected compared with non- infected in (EBNA1 IgG).

4 Discussion

This study found that males outnumbered females in TIDN which is which is similar with other study [11] that showed ratio of females to males was 1:1.4, with a median age of 32 (26- 39) years at biopsy and compared with female patients, male patients exhibited more severe clinical manifestations, including higher levels of serum creatinine, a lower eGFR; heavier proteinuria; and higher proportions of hypertension, hypertriglyceridaemia and hyperuricaemia ($P < 0.05$).Also This result is agreement with most study in the world showed the Male gender is a risk factor for diabetic nephropathy development. Sex hormones influence numerous cellular activities directly or indirectly by influencing the production of different cytokines, growth factors, and vasoactive agents. Estrogen, in particular, regulates genes involved in extracellular matrix metabolism via a receptor-dependent mechanism. Estrogen has a significant impact on transforming growth factor-signaling transduction as well as the renin-angiotensin system. These impacts may lead to changes in renal hemodynamics and affect the course of kidney disease[12]. Male sex has been reported to be a risk factor for the development of diabetic nephropathy, and the factors involved in this sex-specific difference could possibly include lifestyle, diet, differences in glomerular hemodynamics, and direct effects of sex hormones, as well as diabetic duration of more than 10 years as a predictive factor for the development of DN[11]. Also [13] observed that Male sex was associated with the development of macro albuminuria than female. [14] also confirmed that the average incidence of DT1N is high (3% per year) during the first 10 to 20 years after diabetes beginning, and that it takes 15 years for tiny blood vessels in organs such

as the kidney, eyes, and nerves to become affected. It is thought that more than 20%, and possibly up to 40%, of diabetes people may develop CKD .Also[15] discovered that nephropathy progressed in men than in females, and the possible mechanisms behind the renal protective function of the female gender appear to be connected to estrogen hormone. Notably, in other studies, women had a greater prevalence of nephropathy than males. This might be due to the fact that most women were postmenopausal, therefore these findings could be impacted by the loss of estrogen-mediated nephron-protection The current study showed that serum IL-12 levels were significantly higher in T1DN patients than in the control groups. This similar to [16] they observed the Serum IL-12 levels [Geometric mean; p value] were higher in T1DM patients with Macro vascular complication (MVC) [40.4pg/ml ; p 0.001] in comparison to control group[10.89 pg/ml ; p 0.001].Also the result agreement with [17] they found Type 1 diabetes patients had significantly greater serum levels IL12 than control group [1.25pg/ml - 0.1pg/ml ; p 0.00001] respectively. Also [18] they observed the Serum levels of IL-12 demonstrated a significant ($P < 0.001$) increase inT1DM patients as compared to controls (58.02 vs.28.05). [19] discovered that patients with DN had a more obvious Th1 profile recognized by elevated(IL-12) and lower Th2 cytokines (IL-33 and IL-13) showing that DN might be characterized by an increase in Th1 associated with suppression of Th2 response. Current study agreement with Iraq research [20] a high level of IL-12 concentration was discovered in type 1 diabetes patients compared to healthy controls, with a significant difference (p 0.01) between patients (60.15 ± 39.47) and the healthy control group (29.41 ± 21.44). The increased serum IL-12 levels in T1DM were linked to increased pro-insulin secretion. Because IL-12 plays an essential part in immunological response to infections, this cytokine may have a significant role in the etiology of T1DM. In the absence of infection, IL-12-induced auto-reactive T cell responses have been demonstrated to predispose to self-destructive immunity, but the significance of IL-12 alterations in the blood of T1DM patients remains unknown. IL-12 causes of the development of macro vascular problems in the illness [20].[22] confirmed that type 1 diabetes is associated with elevation of IL-12 levels compare to healthy children (23.4 ± 10.79 versus 6.2 ± 2.5) and found this association is more evident in both newly diagnosed and poorly controlled patients indicating a relevant role of IL12 in the pathogenesis of the disease. According to nephropathy patients, this result is consistent with [23] who discovered that infusion of Ang II increased the number of type 1 T helper (Th1) cytokine IFN-γ secreting cells and decreased the number of type 2 T helper (Th2) cytokine IL-4-secreting cells in hypertension patients suffering from nephropathy. [23]found that high levels of proinflammatory cytokines (IL-1,TNF, IL-12, and INF-y) and high levels of ROS increase the development of hypertensive nephropathy. In addition, IL-12 levels in minimal-change nephrotic syndrome (MCNS) and IgAN patients with nephrotic syndrome (NS) were considerably higher than in normal controls [25]. Serum IL-12p40 levels increased significantly in the relapsed group od idiopathic nephrotic syndrome patients and it may be involved in the pathogenesis and recurrence of MCD[26]. [26]who observed elevated IL-12 levels in MCD patients throughout the active period and a recovery to normal levels after the patients entered remission. The current study found the level of IL-12 was significantly elevated in T1DN patients infected with EBNA1 IgG (33.84 ± 4.47) in compare to patients non infected with EBNA1 IgG, this similar to

[27]It was discovered that seroprevalence of EBV anti-VCA IgG antibodies was 100% in autoimmune patients, and that approximately 40.3% of patients had an EBV lytic activity profile, manifesting positivity for the EBNA3C gene in their plasma samples, and that approximately 59.7% of those evaluated with an autoimmunity diagnosis had an EBV viral latency profile. Similarly, [28] conducted an Iraqi research. The presence of significant differences between T1D patients and controls ($P< 0.001$) in the detection of anti EBV IgG antibody suggested that chronic viral infection leads to activated T cells producing more Th-

1 cytokines IL-12, INF-Y, IL-17, and TNF, and that this elevation is associated with disease progression. [29] state that EBV may act as autoimmune initiators in several autoimmune disorders. EBV has several latencies, including type III latency, which has a higher proliferation potential and would facilitate the development of both B and T cell-mediated autoimmunity. It has also been suggested that repeated cytokine productions are achieved by repeated infection of naive B-lymphocytes and proliferation of type III latency cells, which produce inflammatory cytokines that promote autoreactive B cell and T cell proliferation. According to[30,31] elevated levels of IL-12 in Sjögren syndrome when compared to a healthy control group, and that IL-12 expression closely reflects the intracellular event of EBV activation in Sjögren syndrome [32], and may contribute to the Th1 cytokine overexpression seen in this disease. In another type of specific autoimmune disease study, [31]discovered that Crohn's Disease (CD) is associated with an exaggerated T helper 1 cytokine response as manifested by increased production of interleukin-12 (IL-12) and interferon- (IFN-y) and confirmed that Epstein-Barr virus-induced gene 3 (EBI3) encodes a 34-kDa glycoprotein that is 27% similar to the p40 unit of IL-12 and has recently been discovered to be up-regulated in ulcerative colitis (UC). Also[32] , The IL-17 and IL-12 (105.96±16.30; 12.48±3.62 pg/ml) highly increased in Multiple sclerosis patients than control group and this elevation associated with EBV infection and concluded that EBV has a crucial role in the initiation and targeting MS disease, leading to increasing levels of IL-17A [33, 34].

References

1. N. M. Elsherbiny and M. M. H. Al-Gayyar, Cytokine **81**, 15–22 (2016)
2. J. F. dos Santos Haber et al., Biomedicines **11(4)**, 1120 (2023)
3. J. Shang et al., J. Cell. Mol. Med. **23(5)**, 3417–3428 (2019)
4. A. K. H. Lim and G. H. Tesch, Mediators Inflamm. **2012**, (2012)
5. A. S. Awad et al., Kidney Int. **88(4)**, 722–733 (2015)
6. J. P. Driver et al., Diabetes **66(3)**, 710–721 (2017)
7. A. M. Wegeberg et al., Cardiovasc. Diabetol. **21(1)**, 1–10 (2022)
8. C. C. Sorgato et al., J. Oral Pathol. Med. **49(7)**, 693–700 (2020)
9. I.A.J. Aljanaby, H.M.Y. AL-labban, T.H. Hasan, Mohammed Almousawi M. and A.A.J. Aljanaby, Latin American Journal of Pharmacy **42**, 238-240 (2023)
10. I.A.J. Aljanaby, H.M.Y. Al-labban, T.H. Hasan, M.J. Al-anssari, A.A.J. Aljanaby, Neural Cell Adhesion Molecule in Mycobacterium tuberculosis Patients. Latin American Journal of Pharmacy **42**, 184-187 (2023)
11. P. Yang and C. S. Foster, Am. J. Ophthalmol. **156(2)**, 400–406 (2013)
12. J. Neugarten and L. Golestaneh, "Adv. Chronic Kidney Dis. **20(5)**, 390–395 (2013)
13. W. Akram, AL-Kindy Coll. Med. J. **8(2)**, 94–102 (2012)
14. K. Raile et al., Diabetes Care **30(10)**, 2523–2528 (2007)
15. F. M. Hassan, A. A. Khatab, W. M. A. El-Fotoh, and I. N. T. Ganh, Menoufia Med. J. **32(1)**, 305 (2019)
16. T. Toyama et al., BMC Nephrol. **21(1)**, 1–9 (2020)
17. S. Shruthi, V. Mohan, A. Amutha, and V. Aravindhan, Cytokine **8**6, 6–9 (2016)
18. M. Ryba-Stanisławowska, K. Rybarczyk-Kapturska, M. Myśliwiec, and J. Myśliwska, Inflammation **37**, 1513–1520 (2014)

19. A. M. Saud, Int. J. Curr. Microbiol. Appl. Sci. **3(4)**, 260–268 (2014)
20. L. S. Araújo et al., J. Diabetes Mellit. **6(04)**, 230 (2016)
21. M. T. Qasim, "Interleukin-2&Interleukin-12 Levels in Type 1 Diabetes Mellitus Patients".
22. Y. Wen, J. Gu, S.-L. Li, M. A. Reddy, R. Natarajan, and J. L. Nadler, Endocrinology **147(5)**, 2518–2525 (2006)
23. A. A. Alghobashy, D. Shokry, and H. H. Gawish, Egypt. J. Pediatr. Allergy Immunol. **11(1)**, (2013)
24. J. Shao et al., Hypertension **42(1)**, 31–38 (2003)
25. P. A. M. Cavalcante et al., Mediators Inflamm. **2019**, (2019)
26. K. Matsumoto and K. Kanmatsuse **117(2)**, 361–367 (1999)
27. M. Bai et al., Front. Med. **9**, 922193 (2022)
28. J. Wang et al., Hypertension **77(5)**, 1613–1626 (2021)
29. S. A. de S. França et al., Viruses **14(4)**, 694 (2022)
30. A. H. Mohammed, "The roles of Human Cytomegalovirus and Epstein-Barr virus in Type 1 Diabetes Mellitus".
31. L. Zhang, J. Med. Virol. **95(1)**, e28363 (2023)
32. H. Nakamura, T. Shimizu, and A. Kawakami, J. Clin. Med. **9(5)**, 1459 (2020)
33. F. Omata, M. Birkenbach, S. Matsuzaki, A. D. Christ, and R. S. Blumberg, Inflamm. Bowel Dis. **7(3)**, 215–220 (2001)
34. W. S. Abd and R. M. A. Al Kareem, Impact of EBV on multiple sclerosis in some of the Iraqi males: Immunological and molecular study. In AIP Conference Proceedings, AIP Publishing LLC, 2020, p. 20023.