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Detection of Human Parvovirus B19 IgG and DNA in a group of Iraqi children with newly onset type 1 diabetes mellitus

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Abstract:

Background: Type 1 diabetes mellitus is a complex disease that is caused by a combination of factors, one of these factors is viral infection. Some studies assumed that human Parvovirus B19, a virus that can infect red blood cells may play a role in the development of Type 1 diabetes mellitus.

Objective: The aim of this study was to qualitative and quantities detection of Parvovirus B19 IgG and DNA in children with newly onset type 1 diabetes mellitus.

Methods: A total of 50 children with newly diagnosed type 1 diabetes mellitus and 50 non-diabetic children were recruited as controls. Parvovirus B19 was detected using ELISA and real-time PCR technique.

Results: Parvovirus B19 (B19) IgG had been detected in T1DM patient and healthy controls with exposure rate (6%) and (10%) respectively, However results was not statistically significant, while Parvovirus B19 DNA was equal for both groups (2%). Although patients with type 1 diabetes mellitus had a higher $\log_{10}$ viral load but it didn't differed significantly from control.

Conclusion: Current study concluded that parvovirus B19 infection is not considered as risk factor for type 1 diabetes mellitus**.**

Key words: Parvovirus B19, Children, T1DM.

List of abbreviation: B19V= Parvovirus B19, GAD = Glutamic Acid Decarboxylase, IA-2=Islet Antigen-2, T1DM=Type 1 Diabetes Mellitus, ELISA=Enzyme-Linked Immunoassay.

Introduction:

Type 1 diabetes mellitus (T1DM), formerly known as insulin-dependent diabetes mellitus (IDDM), is an autoimmune disease that typically develops in children and adolescents. This disease causes the body to attack its own insulin-producing cells in the pancreas which lead to deficiency of insulin production due to the destruction of pancreatic beta cells and require lifelong insulin administration for survival. T1DM is one of the most common chronic diseases of childhood, accounting for about 10% of all diabetes cases (Atkinson et al., 2014; Li et al., 2014 ; Msekandiana et al., 2020).

T1DM is emerging as a very heterogeneous disease driven by a complex network of multiple factors, including genetic and environmental interactions. (Awadalla et al., 2017; Bilal et al., 2019; Zajec et al., 2022). There is a strong association between viruses and T1DM. Several studies have shown that people with T1DM are more likely to have been infected with certain viruses in the past, such as enteroviruses, mumps virus, and cytomegalovirus (Almahhfoodh et al., 2017; Nekoua et al., 2022). Data from experimental animals as well as in vitro studies indicate that various viruses are clearly able to modulate the development of T1DM via different mechanisms. The earliest observations for the suggestion of virus contribution to T1DM are that the beginning of T1DM sometimes follows acute viral infections (Ibrahim et al., 2021).

Many human viruses have been linked to T1DM for numerous reasons, the B19V was considered a candidate in the pathophysiology of T1DM. Its peak incidence in children, prevalent subclinical presentation, and endemic frequency with irregular outbreak intervals are consistent with the characteristics of an environmental viral agent connected to the development of T1DM (Poblotzki et al.,1996; Boboc et al., 2021). Current study aims to detect B19V in children with newly diagnosed T1DM and compare DNA viral load between patients and apparently healthy children to shed light on its role in causing the disease which could lead to new ways to prevent or delay the onset of T1DM or to improve the treatment of people with T1DM. Also to help in early diagnosis of T1DM which benefit greatly for reducing the sufferings caused due to diabetic complications.

Materials and Methods:

Patients and controls: A case-control study was conducted in Baghdad, Iraq, to investigate the possible link between viral infection and T1DM in children. From October to February 2023, 50 newly diagnosed children with T1DM and 50 non-diabetic controls were included in the study at Central Teaching Hospital of Pediatrics in Baghdad. The research project was approved by the Ethical Committee of Al-Nahrain College of Medicine on 14 November 2022, with approval declaration number 202207/76. The written consent of the child's guardian was obtained prior to conducting the research. The leftover blood samples were collected from 50 children who had just been diagnosed with T1DM and were being attended to the Central

Teaching Hospital of Pediatrics. The diagnosis was confirmed according to the World Health Organization (WHO) criteria, which include random blood sugar (RBS) and hemoglobin A1c (HbA1c) levels.

Specimens collection: Five milliliters of the remaining sample was divided into two parts. Three milliliters was placed in a sterile gel tube and allowed to clot, the serum was then separated by centrifugation at 4000 rpm for 15 minutes. The serum was used to estimate the levels of insulin, anti-glutamic acid decarboxylase antibodies (GADA 65), and anti-islet antigen antibody (IA-2A). The remaining 2 milliliters of blood was kept in Ethylene Diamine Tetra acetic Acid (EDTA) for estimating HbA1c and viral DNA extraction.

DNA extraction: Viral Nucleic Acid Extraction Kit II (Promega, USA) was used to isolate and purify DNA from blood samples. The procedure was followed according to the manufacturer's instructions.

Viral diagnosis: Parvovirus B19 (B19) will be diagnosed by method. Enzyme-Linked Immunoassay (ELISA) technique will be used to detect IgG in serum against the virus. Also PVB19 DNA amplification was done by Real-Time PCR according to the manufacturer guide lines (Sacace, Italy), target region is structural protein (VP1) gene. Thermal profile of the PB19V qRT-PCR run was included one cycle of initial denaturation at 95°C for 15 min, then five cycles of denaturation, annealing, and extension at 95°C (5 sec), 60°C (20 sec) and 72°C (15 sec). Then repeated for further 40 cycles of denaturation, annealing, and extension with temperature at 95°C (5 sec), 60°C (30 sec) and 72°C (15 sec) with fluorescent signal detection at FAM,JOE/HEX/Cy3 channel.

Estimation the level of insulin: Insulin ELISA Assay was used for the quantitative measurement of human insulin in serum of patients and controls.

Interpretation of the results: Parvovirus B19V IgG the calculated absorptions for the patient and control serum were compared with the cut off value. The cut off value = absorbance of negative control + 0.15, If the absorbance of the sample is equal or higher than the cut off value, the test sample is considered positive, otherwise the test sample is considered negative, while PCR amplification products were interpreted using the qualitative analysis function of the real-time PCR instrument, this function by crossing of the fluorescence curve with the threshold line. Samples that cross the threshold line are considered positive for the target DNA, while samples that do not cross the threshold line are considered negative. The standard curve was plotted using the data from the known standards (QS1 and QS2). The Y-axis of the standard curve is the cycle threshold (CT) value, and the X-axis is the log of the starting quantity of DNA. The concentration of PVB19 DNA in the positive samples and control was calculated using the following formula:

$$\frac{B19V\ DNA\ copies/specimen}{IC\ DNA\ copies/specimen} \times 8.2 \times 10^4\ \text{copies/ml} = Copies\ DNA\ B19V/ml$$

Concerning insulin, (GADA 65), and (IA-2A), known concentrations are plotted on the log scale (x-axis) and the log scale (y-axis), respectively. The concentration of the marker in tin sample is determined by plotting the sample's O.D. on the Y-axis and then its original concentration is calculated by multiplying the dilution factor.

Statistical Analysis: The statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) software version 25.0 and Microsoft Excel 2013. Categorical data were presented as counts and percentages Chi-square test (χ2-test) was used to describe the association between categorical variables. Normally distributed data were presented as mean and standard deviation and analyzed using sample t-test.

Non-normally distributed data were presented as median and range and analyzed using the Mann Whitney U test (for two group comparisons). In all statistical analyses, the level of significance (P value) was set at ≤ 0.05. The results were presented in tables and/or graphs.

Results:

Study population:

Table (1): Study population demographics and clinical features

Variables	T1DM Patients (n=50)	Controls (n=50)	P-value
Age, years			
Mean±SD	8.08±4.65	7.88±4.42	0.974*
Median	8	8	
Range	< 1-15	1-15	
Sex			
Male	20 (42.6%)	27 (57.4%)	0.161**
Female	30 (56.6%)	23 (43.4%)	
Weight, kg			
Mean±SD	28.88±14.02	33.24±18.40	0.381*
Median	27	31	
Range	8-62	8-78	
Residence			
Urban	35 (70%)	48 (96%)	< 0.001**
Rural	15 (30%)	2 (4%)	
Family history			
No	18 (36%)	37 (74%)	< 0.001**
Yes	32 (64%)	13 (26%)	
Disease duration, day			
Mean±SD	3.98±3.30	----------	----------
Range	1-23		
Insulin using			
Yes	31 (62%)	----------	----------
No	19 (38%)		
Type of insulin			
Self-titrated	13 (43%)	----------	----------
Premixed	17 (56.7%)		

**Mann Whitney U test, ** x2- test

Biochemical tests related to T1DM: RBS and HbA1c levels in patients were significantly higher (217.20±87.7 mg/dl and 10.27±1.89, respectively) than in controls (88.7±7.3 mg/dl and 4.92±0.5, respectively), with significant differences (P<0.05). Furthermore, patients exhibited lower insulin levels than controls

(1.99±0.66 mU/l versus 2.60±1.52 mU/l), with a statistically significant difference (P=0.001) (table 2).

Table (2): Biochemical tests related to study population

Tests	Patient population Mean±SD (n=50)	Control population Mean±SD (n=50)	*P* value*
RBS, mg/dl	217.20±87.7	88.7±7.3	**< 0.001***
HbA1c, %	10.27±1.89	4.92±0.5	**0.004***
Insulin, pg/ml	1.99±0.66	2.60±1.52	**< 0.001****
Normal values: RBS <200 mg/dl, HbA1c 5.7-6.4 %			

*Mann Whitney U test **Student t- test

Figure (1) and (2) shows that the median levels of (Anti-IA2) and (GAD65) in patients were 43.7 pg/ml and 27.7 ng/ml, respectively, compared with 15.3 pg/ml and 13 ng/ml respectively, in control with highly significant differences (*P*<0.001).

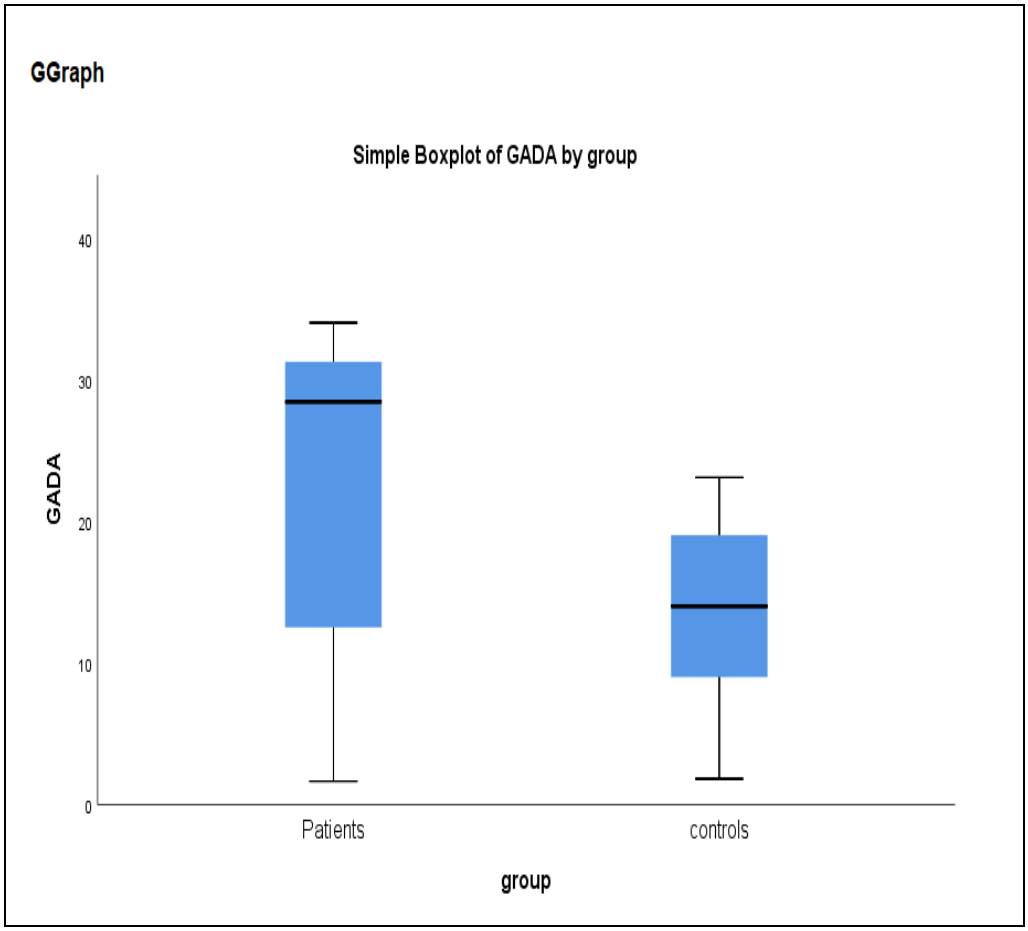

Figure (1): T1DM and controls -Related GAD

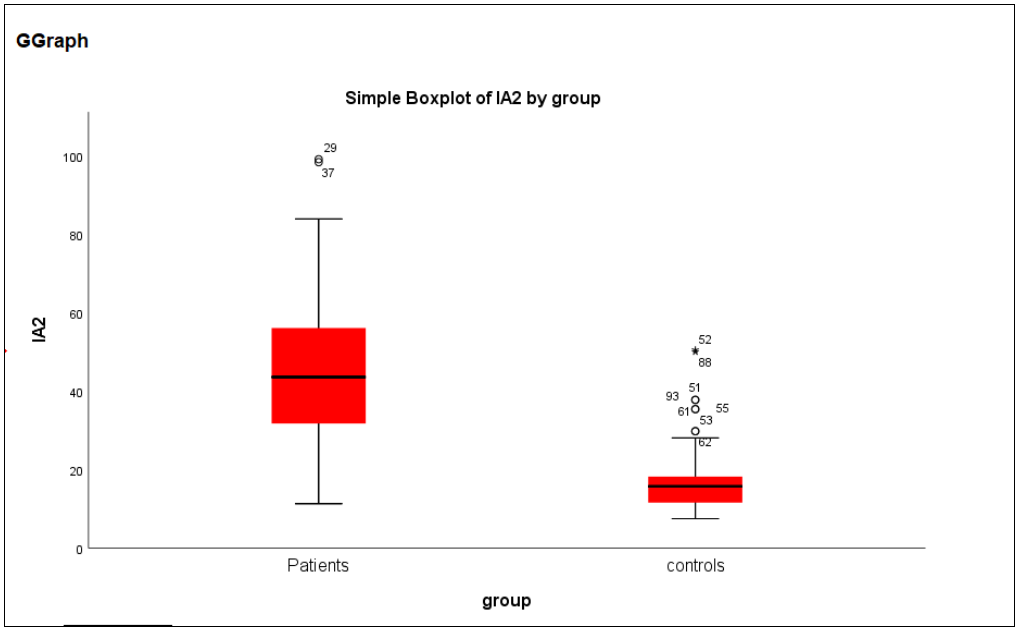

Figure (2): T1DM and controls -Related GAD

Detection of anti-Parvovirus (B19) IgG antibody

The differences in occurrence of PVB19 IgG between patients with T1DM and controls are not significant, (6%) of patients tested positive for anti-parvovirus (B19) IgG antibodies, and (10%) of control group tested positive ($P > 0.05$) as shown in figure (3).

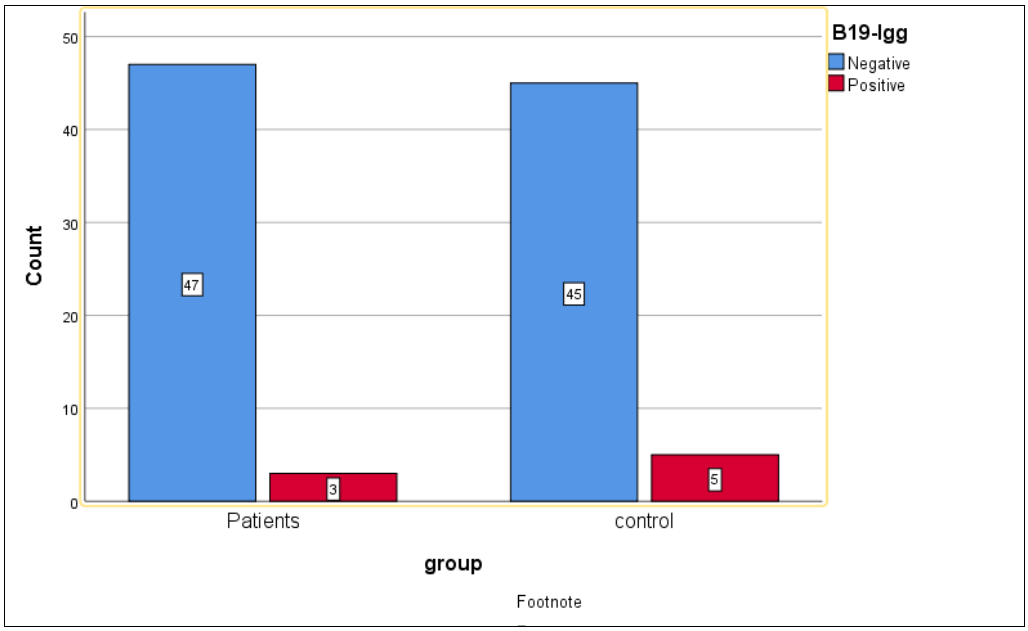

Figure (3): Frequency of anti-Parvovirus (B19V) IgG antibodies in T1DM patients and controls

laboratory parameters (Anti-IA2), (GAD65) and Insulin showed a non significant association with anti-Parvovirus IgG antibodies for patients and controls.

Table (3): Association of anti-Parvovirus B19 IgG antibodies positivity with autoantibodies related to patients and controls .

Laboratory Parameters	Controls			Patients		
	IgG positive (5)	IgG negative (45)	Pvalue	IgG positive (3)	IgG negative (47)	Pvalue
Anti-IA2, pg/ml Median Range	16.05 12.7-17.39	15.3 7.4-25.8	0.109*	72.6 30.9-83.8	43.87 11.3-71.4	0.184*
GAD, ng/ml Median Range	10.10 8.5-13.10	13.0 1.8-23.1	0.263*	29.39 11-31.5	27.7 1.6-32.2	0.984*
Insulin, mU/L Median Range	2.75 1.1-4.9	2.63 0.1-8.4	0.771*	2.05 0.7-2.5	1.94 0.6-3.5	0.444*

***Mann Whitney U test**

PB19V Viral load among patients and controls: Quantitative real-time PCR was used for detection of viral DNA in whole blood samples using specific set of primers. The results are depicted in figure (3) and figure (4). Only one of the children among control (2%) was positive, compared to one (2%) of patients with no significant difference (P> 0.05).

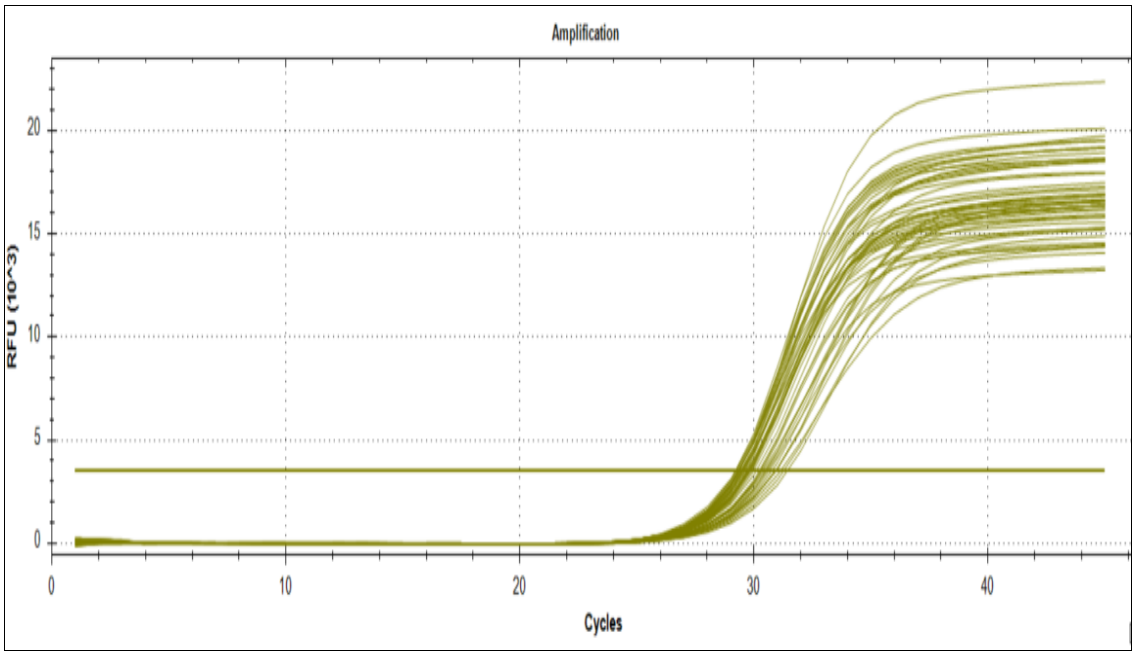

Figure (4): The results of qPCR for internal control for parvovirus viral DNA detection.

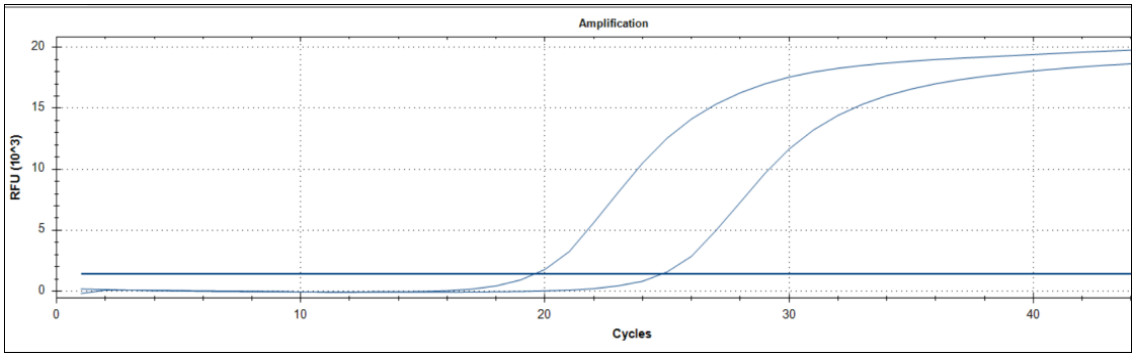

Figure (5): Parvovirus DNA amplification curves obtained during real-time PCR assays with blood samples from T1DM patients and controls. The relative fluorescence units (RFU) are plotted against threshold cycle (Ct) number. The Ct variation was observed among patients, which yielded homogeneous values below 35. Values above 40 were considered negative.

The result qPCR was considered as the golden standard for viral positivity, the viral load was calculated in copies/ml based on specific formula. Because the relatively high values of viral load, these values were transformed into $\log_{10}$ formula to normalize the distribution of the data. Figure (5) shows mean $\log_{10}$ viral load in patients and controls that is considered positive for qPCR. Patients with T1DM had a higher mean $\log_{10}$ viral load (16±113.13 copies/ml) and didn't differed significantly (P= 0.989) from that of control(12±84.85 copies/ml).

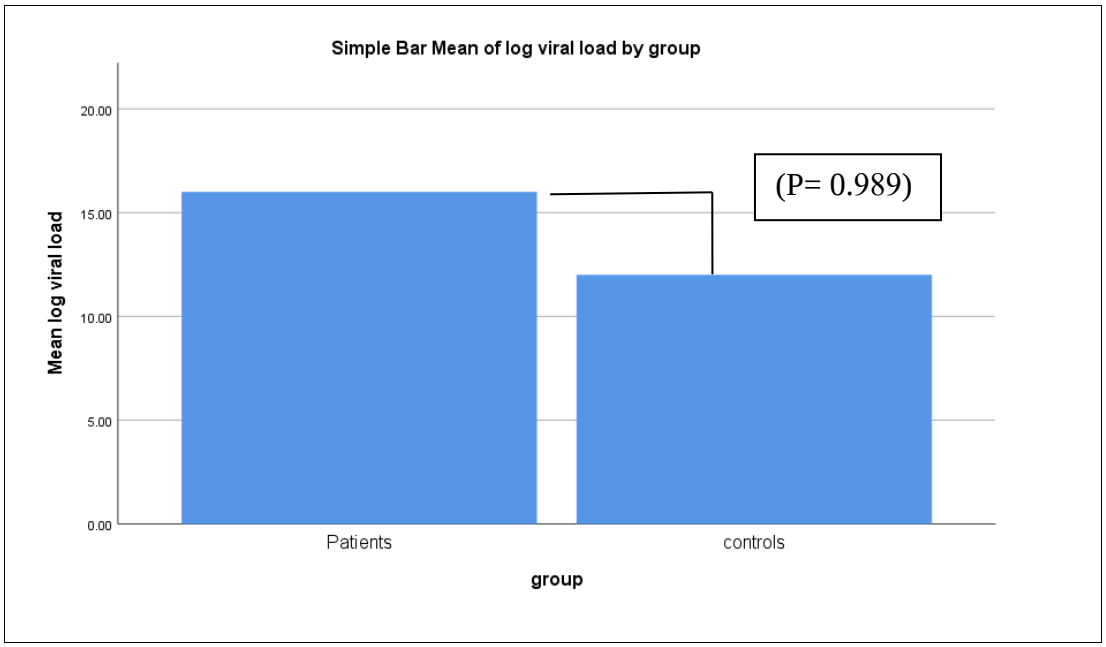

Figure (6): Parvovirus B19 viral load in positive patients and controls using qPCR.

Discussion:

Viral infections have long been suspected as a possible environmental trigger for T1DM. However, the evidence for this association is still largely circumstantial. There are a number of viruses that have been linked to T1DM, including

enteroviruses, rotavirus, mumps virus, and cytomegalovirus. Enteroviruses are the most well-studied such as Coxsackievirus have been shown to induce or accelerate T1DM, also several studies considered a link between B19V infection and the pathogenesis of T1DM (Hassan et al., 2022; O'Bryan et al 2005).

β-cell autoimmunity is the process by which the bodys immune system attacks and destroys the beta cells in the pancreas. The presence of autoantibodies is a strong predictor of future development of T1DM. In fact, children who have autoantibodies to beta cell antigens are more likely to develop type 1 diabetes autoantibodies such as GAD65, anti-IA-2, ICA, and ZnT8. Autoantibodies are used as crucial diagnostic tools, also it is necessary to differentiate between diabetes types (Zamanfar et al.,2020).

The present study revealed that the presence of B19 IgG did not differ significantly between the diabetic and the control groups [three of 50 (6%) diabetic subjects, five of 50 (10%) healthy controls]. Also there was no relationship between this virus and the presence of diabetic autoantibodies as shown in figure (1), (2), (3) and table (3).

Also Current study found that frequency of B19 V DNA did not differ significantly between diabetes and control groups [one of 50 (2%) diabetic patients, one of 50 (2%) healthy controls ($P > 0.05$)], also Patients with T1DM had a higher mean $\log_{10}$ viral load (16±113.13 copies/ml) and didn't differed significantly ($P = 0.989$) from that of control (12±84.85 copies/ml) as shown in figure (4), (5) and (6). This means that the presence of B19V DNA in a child's blood does not appear to increase their risk of developing T1DM. The present finding is similar with other studies such as O'Brayan et al. (O'Brayan et al., 2005) in Pennsylvania. Also in Iraqi population a cross sectional study includes 45 patient with T1DM that showed no significant association between PB19V with T1DM (Mohammed et al., 2021). However, results of this study does not rule out the evidence association between B19V infection and the development of T1DM because this study was limited by its small sample size only 50 patients and 50 controls, so, there is less power to detect a difference, also the fact that it was conducted in a single geographic location. Further research is needed to confirm these findings and to determine whether B19V infection may play a role in the development of T1DM.

Many possible mechanisms by which viruses could trigger T1DM, first by damaging pancreatic β cells, the cells in the pancreas that produce insulin. This damage could lead to the release of antigens from the β cells, which could then trigger an autoimmune response against the β cells (Nadimpalli et al., 2015).

Another possibility is that viruses could disrupt the immune system in a way that makes it more likely to attack the β cells. For example, viruses could suppress the activity of regulatory T cells, which normally help to keep the immune system in check. It is also possible that viruses could act indirectly to promote T1DM. For example, they could increase the risk of other infections, which could then damage the β cells or disrupt the immune system (Malbora et al., 2017). Parvovirus B19 also can lead to type 1 diabetes through molecular mimicry, this occurs when the virus has

amino acid sequences that are similar to the extracellular domain of IA-2 found in insulin-producing cells pancreatic β cells. This can lead to the body's immune system mistakenly attacking the body's own cells. Molecular mimicry may also be involved through induction of virus-specific B and T cells by macrophages presenting viral peptides (O'Bryan et al., 2005). Another possible mechanism is through bystander activation. This occurs when the virus might infect non-β cells in the pancreas, causing inflammation in the area. The release of cytokines and chemokines from these inflammatory sites can be harmful to β cells (Chisaka et al., 2003). Parvovirus B19 may also induce activation of macrophages through TLR triggering, resulting in the secretion of pro-inflammatory mediators (e.g. IL-1, IL-6, Il-8,MCP-1, and TNF-α) (Fujinami et al.,2006).

Furthermore, B19V may infect thymic epithelial cells, resulting in disrupted maturation of T-cells with autoimmune characteristics. Pro-inflammatory mediators may enhance autoimmunity by increasing autoreactive T-cell responses by selective deletion/impairment of regulatory T cells (Malbora et al.,2017). It is likely that a combination of these mechanisms is involved in the development of T1DM in people who are infected with B19V. More research is needed to better understand the role of B19V in T1DM and to develop ways to prevent or treat the disease (Ellerman et al., 1996).

Conclusion: The current study showed that PVB19 not associated with the onset of T1DM in a samples of Iraqi children, further research is needed to confirm this finding and to understand the role of viral infection in T1DM development.

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Author contribution: Khudair performed the laboratory works, the statistical analysis, and wrote the draft of this paper as part of her PhD thesis, Prof. Dr. Al-Shuwaikh designed, supervised this work and arranged the final version of this manuscript, Dr. Abdoun contributed to sample collection and clinical aspects of this work. The final version of this manuscript was read and approved by all the authors

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References:

1. Almahfoodh D, Alabbood M, Alali A, Mansour A. Epidemiology of type 1 diabetes mellitus in Basrah, Southern Iraq: A retrospective study. diabetes research and clinical practice. 2017;133:104-8. DOI:10.1016/j.diabres.2017.09.001.
2. Atkinson MA, Eisenbarth GS, Michels AW. Type 1 diabetes. The Lancet. 2014 ;383(9911):69-82. DOI:10.1016/S0140-6736(13)60591-7
3. Awadalla, N. J., Hegazy, A. A., El-Salam, A., & Elhady, M. Environmental factors associated with type 1 diabetes development: a case control study in Egypt. International journal of environmental research and public health. 2017; 14(6): 615. DOI:10.3390/ijerph14060615

4. Boboc AA, Novac CN, Ilie MT, Ieșanu MI, Galoș F, Bălgrădean M, Berghea EC, Ionescu MD. The impact of SARS-CoV-2 pandemic on the new cases of T1DM in children. A single-centre cohort study. Journal of personalized medicine. 2021 Jun 13;11(6):551. DOI:10.3390/jpm11060551.
5. Chisaka H, Morita E, Yaegashi N, Sugamura K. Parvovirus B19 and the pathogenesis of anaemia. Rev Med Virol. 2003;13(6):347–59. DOI:10.1002/rmv.395
6. Ellerman KE, Richards CA, Guberski DL, Shek WR, Like AA. Kilham rat virus triggers T-cell–dependent autoimmune diabetes in multiple strains of rat. Diabetes. 1996;45(5):557-62. DOI:10.2337/diab.45.5.557.
7. Fujinami RS, Von Herrath MG, Christen U, Whitton JL. Molecular mimicry, bystander activation, or viral persistence: Infections and autoimmune disease. Vol. 19, Clinical Microbiology Reviews. 2006. p. 80–94. DOI:10.1128/cmr.19.1.80-94.2006
8. Hassan HG, Al-Shuwaikh AM, Kareem R. Association of Anti-Coxsackievirus-B IgG With IFN-α, IFN-β, IL-6 and TNF-α in Children With Type 1 Diabetes. Journal of Natural Science, Biology and Medicine. 2022 Jan;13(1). DOI:10.4103/jnsbm.JNSBM_13_1_8
9. Ibrahim IE, Fathy M, Ghada Ma, Nancy M. Autoimmunity and Pathogenesis of Type 1 Diabetes. The Medical Journal of Cairo University. 2021;89:1341-7. Doi:10.21608/mjcu.2021.185045. DOI: 10.21608/mjcu.2021.185045.
10. Li M, Song LJ, Qin XY. Advances in the cellular immunological pathogenesis of type 1 diabetes. Journal of cellular and molecular medicine. 2014;18(5):749-58. DOI:10.1111/jcmm.12270.
11. Malbora B, Saritas S, Ataseven E, Belen B, Alparslan C, Yavascan O, et al. Pure red cell aplasia due to parvovirus B19: Erythropoietin-resistant anemia in a pediatric kidney recipient. Exp Clin Transplant. 2017;15(3):369–71. DOI: 10.6002/ect.2016.0263.
12. Mohammed A. Effect of Parvovirus B19 in Diabetes Mellitus Type 1. In Proceedings of 2nd International Multi-Disciplinary Conference Theme: Integrated Sciences and Technologies, IMDC-IST 2021, 7-9 September 2021, Sakarya, Turkey 2022 Jan 26. DOI:10.4108/eai.7-9-2021.2314785
13. Msekandiana, A., Chigayo, G., Chiume, S., Jaulani, A., & Msuya, L. Complications and Glycaemic Control of Type 1 Diabetes Mellitus amongst Children Aged 5 to 19 Years Attending Diabetic Clinic at Kamuzu Central Hospital In Malawi. Int J Diabetes Clin Res. 2020;7: 117. DOI: 10.23937/2377-3634/1410117
14. Nadimpalli SS, Miller RS, Kamath VM, Farkouh CR, Nhan-Chang C-L, Rathe JA, et al. Congenital parvovirus B19 infection: persistent viremia and red blood cell aplasia. In: Open Forum Infectious Diseases. Oxford University Press; 2015. p. ofv049. DOI:10.1093/ofid/ofv049
15. Nekoua MP, Alidjinou EK, Hober D. Persistent coxsackievirus B infection and pathogenesis of type 1 diabetes mellitus. Nature Reviews Endocrinology. 2022;18(8):503-16. DOI:10.1038/s41574-022-00688-1

16. O'Brayan TA, Beck MJ, Demers LM, Naides SJ. Human parvovirus B19 infection in children with new onset Type 1 diabetes mellitus. Diabet Med. 2005;22(12):1778-9. DOI: 10.1111/j.1464-5491.2005.01708.x.
17. Von Poblotzki AN, Gerdes C, Reischl U, Wolf H, Modrow S. Lymphoproliferative responses after infection with human parvovirus B19. Journal of virology. 1996;70(10):7327-30. DOI:10.2174/157339706776876099
18. World Health Organization. Laboratory diagnosis and monitoring of diabetes mellitus. World Health Organization; 2003. https://apps.who.int/iris/handle/10665/42642
19. Zajec A, Trebušak Podkrajšek K, Tesovnik T, Šket R, Čugalj Kern B, Jenko Bizjan B, Šmigoc Schweiger D, Battelino T, Kovač J. Pathogenesis of type 1 diabetes: established facts and new insights. Genes. 2022;13(4):706. DOI:10.3390/genes13040706.
20. Zamanfar D, Aarabi M, Amini M, et al. Prevalence of autoantibodies in type 1 diabetes mellitus pediatrics in Mazandaran, North of Iran. J Ped Endocrinol Metab. 2020; 33(10): 1299-305. DOI: 10.1515/jpem-2019-0396.
21. Zamanfar D, Aarabi M, Amini M, Monajati M. Prevalence of autoantibodies in type 1 diabetes mellitus pediatrics in Mazandaran, North of Iran. Journal of Pediatric Endocrinology and Metabolism. 2020 Aug 17;33(10):1299-305. DOI: 10.1515/jpem-2019-0396.