

Raised levels of Interleukin-8 and Myeloperoxidase in patients with recent onset type 1 diabetes

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ABSTRACT

Low-grade inflammation and influx of cytokines have been associated with increased pancreatic β -cell damage and progression of type 1 diabetes (T1D). Circulating interleukin-8 (IL-8) has been reported to be elevated in adults and children with T1D, compared to healthy controls. The major aim of the present study was to measure IL-8 and IL-8 receptor expression levels in the pancreas, and IL-8 and myeloperoxidase (MPO) levels in serum, in people with and without recent-onset T1D and investigate possible relationships with metabolic parameters.

Pancreas samples obtained within 2 years of diagnosis of T1D and from non-diabetic controls were from an established diabetes biobank. Serum was collected from subjects with newly diagnosed T1D within their first year of disease onset (n=42), along with healthy controls (n=11). Patient clinical characteristics were collected including: age, gender, body mass index (BMI), disease duration, HbA1c, blood glucose levels, and C-peptide. Serum IL-8 and myeloperoxidase were measured with commercial enzyme-linked Immunosorbent assay (ELISA) kits.

Significant increases in IL-8 and IL-8 receptor gene expression were observed in pancreatic sections from individuals with recent-onset Type 1 diabetes when compared to healthy donors. T1D patients showed significantly increased serum IL-8 levels compared to controls. When assessing the differences between MPO levels in recent onset T1D patients and controls, there was a significantly higher MPO level in T1D than controls (median (IQR), 95327 [55718 – 168974] vs 42729 [24147 – 95327] pg/mL, p-value= 0.0349, respectively). Patients with T1D and IL-8 levels > 62 pg/mL presented with significantly higher levels of both HbA1c and blood glucose levels compared with subjects with IL-8 levels $\leq$ 62 pg/mL (HbA1c; mean $\pm$ S.D, 11.28 ± 2.7 vs 8.9 ± 1.91 , p-value= 0.0154, and, blood glucose; mean $\pm$ S.D, 383.0 ± 137.4 vs 258.8 ± 67.55 , p-value= 0.0151, respectively).

The results of this study suggest that IL-8 and IL-8 receptor levels are increased in the pancreas in T1D with similar increases in IL-8 and MPO in the circulation. Furthermore, this study demonstrated a clear trend for increased HbA1c levels and IL-8 in patients with high circulating IL-8 levels compared to patients with normal IL-8 values.

INTRODUCTION

Type 1 diabetes (T1D) is an autoimmune disorder characterised by the selective destruction of the insulin-producing pancreatic beta-cells (β -cells) predominantly mediated by autoreactive T cells, monocytes and inflammatory cytokines, leading to insulin depletion and hyperglycemia [1, 2]. The heterogeneity of T1D leads to difficulty in determining the pathophysiology and progression of the disease; however low-grade inflammation and influx of cytokines have been associated with increased β -cell damage and the progression of T1D [2].

In fact, studies on non-obese diabetic mice (NOD mice) have demonstrated that inducing alterations of cytokine levels and functions can play a therapeutic role in T1D, resisting immune cell facilitated β -cell damage [3]. Moreover, research is ongoing to identify alternatives to broadly acting immunosuppression drugs that can block beta-cell autoimmunity through specific interactions, such as inhibiting receptor signaling to regulate peripheral tolerance, restoring imbalance between T_{reg} and Th17 cells, or administering IL-2 variants that preferentially activate T_{reg} cells, and the CTLA-4–CD28 pathway. Thus far, therapies directed at obtaining favorable effector-to-regulatory T-cell ratios have shown the most promise [4], but the role of the innate immune system in pancreatic dysregulation has not been fully elucidated. Past literature has determined the presence of resident neutrophils within the pancreatic β -cell microenvironment [5]. Interleukin-8 (IL-8; CXCL8) is a pro-inflammatory chemokine and a powerful neutrophil chemotactic factor. IL-8 is expressed in various cells, including macrophages, neutrophils, fibroblasts and epithelial cells [6]. Macrophages release IL-8 at the site of an injury, where it recruits and activates neutrophils by interacting with IL-8 receptors CXCR1 and CXCR2. Activated neutrophils release myeloperoxidase (MPO). Increases in the concentration of circulating IL-8 have been associated with several autoimmune disorders [7]. There is evidence that IL-8 serum levels may be elevated in adults [8] and children [9] with T1D, compared to healthy controls, although this was not confirmed in a meta-analysis of 14 studies [10].

IL-8, but not the inflammatory agents IL-6 or TNF- α , was elevated in adolescents with established T1D, which showed a further association with poor glycemic control [11]. This may suggest a role for IL-8 beyond acute inflammation in this setting. *In vitro* experiments have revealed that IL-8 transcription is induced by hyperglycemia [12]. Little is known about the role of IL-8 in T1D; hence the major aim of the present study was to measure levels of IL-8 in serum and pancreas in recent-onset T1D and to investigate a possible relationships of serum IL-8 with metabolic parameters.

MATERIALS AND METHODS

Patient samples

IL-8 (*CXCL8*) and IL-8 receptor (*CXCR1*) gene expression in human pancreas was analysed in samples of paraffin-embedded tissue from the Exeter Archival Diabetes Biobank (<https://pancreatlas.org/datasets/960/overview>). Ethical approval for research on the Biobank was granted by the West of Scotland Research Ethics Service (REC reference 15-WS-0258). Pancreas samples from 29 type 1 diabetes cases (13 male, mean age 12 years, range 1–23 years) who died within 2 years of diagnosis were suitable for this study, together with samples from 7 non-diabetic pancreas donors (mean age 16 years, range 2–32 years) as non-diseased controls.

Analysis of serum IL-8 levels was conducted at the Endocrinology unit of the Policlinic Campus Bio-Medico University of Rome, Italy. During this study, individuals newly diagnosed with T1D within their first year of disease onset (N=42) were recruited, along with healthy controls (N=11). Patient clinical characteristics were collected, and binomial data was presented as percentages (%), normally distributed data as mean $\pm$ standard deviation (S.D) and not normally distributed as median and inter-quartile range (IQR). Clinical characteristics collected included age, gender, body mass index (BMI), disease duration, HbA1c, blood glucose levels, and C-peptide.

Serum samples were collected from patients with signed consent, patients too young to sign informed consent had consent given by their parents or guardians. Samples were collected via

venipuncture from the patients, and then centrifuged at 6000 rpm for 10 minutes. Once centrifuged, the serum was collected and placed in either cryovials or Eppendorfs and stored at -20°C for short term storage or -80°C for long term storage until used.

Gene expression NanoString nCounter profiling of human pancreas tissue

Pancreatic levels of IL-8 were extracted from data obtained in a previously published study [13] of inflammatory gene expression in samples of paraffin-embedded human pancreas from the Exeter Archival Diabetes Biobank Sections (4 µm thick) cut from the tissue blocks were used for extraction of total RNA for gene expression analysis. RNA extraction and gene expression profiling using the nCounter Human Autoimmune Profiling Panel (NanoString Technologies, Inc.) on the NanoString nCounter FLEX platform was performed and processed as described [13].

Assessment of proinflammatory cytokines

Serum IL-8 and myeloperoxidase were measured with commercial enzyme-linked Immunosorbent assay (ELISA) kits (ThermoFisher Scientific, cat. number BMS204-3), according to the manufacturer's instructions. For the purpose of this study, patients were divided into groups with high or low serum IL-8 levels, using the upper limit of normal (62 pg/mL [7]) as the cut-off.

Statistical Analyses

All statistical analyses included in this study were performed on GraphPad Prism (GraphPad Prism version 10.0.0 for Windows, GraphPad Software, Boston, Massachusetts USA)

RESULTS

Pancreatic IL-8 and IL-8 receptor expression in Type 1 diabetes and non-diabetic controls

To assess if gene expression levels of *CXCL8* (IL-8) and *CXCR1* (IL-8 receptor) were altered in individuals with and without Type 1 diabetes in the target organ, the pancreas, previously published NanoString nCounter platform datasets [13] were interrogated (Fig 1). Expression of both the ligand,

CXCL8 and the receptor, *CXCR1*, were significantly increased in the pancreas of individuals with Type 1 diabetes (n=29) when compared to those without diabetes (n=7).

Cytokine Levels between patients with T1D and healthy controls

To determine whether increased IL-8 expression in the diabetic pancreas was reflected by changes in the circulation, serum samples from people with and without Type 1 diabetes were analysed for circulating IL-8 and the leukocyte activation marker, MPO. The demographic patient characteristics of N=42 patients within their first year of T1D diagnosis were included in the study are presented in **Table 1**. Patients were initially divided into two groups depending on IL-8 levels, either ≤ 62 pg/mL or > 62 pg/mL. Based on this, n= 10 patients had IL-8 levels ≤ 62 pg/mL and n=32 patients had an IL-8 concentration > 62 pg/mL. There appeared to be no significant difference between age, BMI, C-peptide or MPO levels between groups (p-value= 0.6424 vs 0.0583 vs 0.988 vs 0.9282, respectively). However, there were significant differences between HbA1c (p-value= 0.0154), blood glucose levels (0.0151), and TNF α (0.034) between the groups.

Upon assessing IL-8 levels between recent onset T1D and healthy controls, T1D patients appeared to have significantly increased IL-8 levels compared to the healthy controls included in the study (median (IQR), 112.0 [64.74-311.8] vs 43.05 [30.94-49.70] pg/mL, p-value= 0.002, respectively) (**fig. 2A**). When assessing the differences between myeloperoxidase (MPO) levels in recent onset T1D patients and healthy controls, there appeared to be a significantly higher MPO level in T1D than healthy controls included in this study (median (IQR), 95327 [55718 – 168974] vs 42729 [24147 – 95327] pg/mL, p-value= 0.0349, respectively) (**fig. 2B**).

The association of IL-8 levels with MPO levels in both recent onset T1D and healthy controls was assessed in **figure 3**. There appeared to be a strong positive correlation with increased IL-8 levels and MPO levels in recent onset T1D patients ($r = 0.312$, p-value= 0.024) (**fig 3A**). Moreover, there was

no significant correlation found between IL-8 levels and MPO levels in the healthy controls in this study ($r = -0.273$, $p\text{-value} = 0.418$).

IL-8 levels $\leq$ or > 62 pg/mL were assessed against HbA1c (% **fig. 4A**) and blood glucose levels (**mg/dL**, **fig. 4B**). The figure shows that patients with T1D and IL-8 levels > 62 pg/mL presented with significantly higher levels of both HbA1c and blood glucose levels compared with individuals who had IL-8 levels ≤ 62 pg/mL (HbA1c; mean $\pm$ S.D, 11.28 ± 2.7 vs 8.9 ± 1.91 , $p\text{-value} = 0.0154$, and, blood glucose; mean $\pm$ S.D, 383.0 ± 137.4 vs 258.8 ± 67.55 , $p\text{-value} = 0.0151$, respectively).

The association of IL-8 levels and demographic characteristics were then assessed in $N=42$ recent onset patients with T1D (**fig. 5**). Upon correlation of age (**fig 5C**) and C-peptide levels (**fig. 5D**) with IL-8 levels in the total patient population, there appeared to be no significant correlation ($r = -0.13$, $p\text{-value} = 0.20$, and $r = -0.17$, $p\text{-value} = 0.14$, respectively). However, upon determining the correlations of IL-8 levels in the patient population with HbA1c (**fig 5A**) and BMI (**fig 5B**), there was a trend towards a positive correlation with HbA1c against IL-8 and a negative correlation of BMI against IL-8 ($r = 0.23$, $p\text{-value} = 0.072$, and $r = -0.26$, $p\text{-value} = 0.053$, respectively).

DISCUSSION

T1D is a chronic condition characterized by a pro-inflammatory state that is suggested to mediate the destruction of the pancreatic β -cells, leading to the depletion of β -cell mass and little to no insulin production and secretion [14]. The results of this study show significant increases in IL-8 gene expression in the pancreas in T1D, an observation that is supported by the finding that levels of IL-8 and MPO are elevated in the circulation of people diagnosed with recent onset T1D. The increases in IL-8 are consistent with a study by Dakovic et al., that showed significantly higher salivary levels of IL-8 in T1D when compared to healthy controls [15]. Additionally, Van Sickle et al., showed that

IL-8 levels, but no other inflammatory markers, were elevated in adolescents with T1D who had poor glycemic control [11].

Recent research has implicated the role of the innate immune system in the development of T1D. Unknown environmental factors, such as viral infections, have been suggested to induce an innate immune response and the recruitment of pro-inflammatory cytokines in individuals genetically predisposed to T1D [16]. Both IL-8 and MPO are essential cytokines released during the innate immune reaction. MPO is a key enzyme stored in the lysosomal azurophilic granules of neutrophils [17], while IL-8 is a chemoattractant, and recruits' neutrophils to the site of inflammation [6]. Hence, the co-elevation of these two cytokines may suggest a more prominent role of neutrophilic mediated β -cell destruction in these patients.

Furthermore, this study demonstrated a trend with increased HbA1c levels and IL-8, showing that patients with IL-8 levels > 62 pg/mL had significantly higher HbA1c levels and blood glucose levels compared to patients with IL-8 ≤ 62 pg/mL. This finding suggests that increased HbA1c and blood glucose levels may be attributed to the dysregulated inflammatory response seen in T1D. This is further corroborated by elevated HbA1c having been previously associated with increased inflammatory markers such as c-reactive protein and albumin levels, not only within patients with diabetes, but also in patients without diabetes and elevated HbA1c levels [18, 19]. This may suggest that the excessive elevation of inflammatory cytokines plays a role in dysregulating the pancreatic beta cells, and thus in turn impacting metabolic control and glucose homeostasis, and thus the more severe the progression of T1D.

In addition, studies have demonstrated that demographic characteristics such as BMI are significantly associated with IL-8 levels in patients with subclinical hypothyroidism and new onset type 2 diabetes [20]. Conversely, within this study there appeared to be a negative trend with elevated BMI and IL-8, this is consistent with the findings of a study that compared adipokine and

inflammatory cytokine levels. This study assessed the adipokine and inflammatory cytokine profiles of 32 lean and 18 obese children with new-onset T1D and did not reveal significant differences in IL-8 between these groups [21].

The elevation of IL-8 and MPO may suggest that there is overt crosstalk between both the innate and adaptive immune systems in the pathophysiology of T1D. Within T1D, diagnosis and progression are monitored by the detection of autoantibodies directed towards native pancreatic β -cell antigens, indicating that a breakdown in immune tolerance plays a key role in the pathogenesis of T1D [22]. However, new studies are suggesting that the β -cells themselves are the major players in their own demise. These studies state that the β -cells are ill-equipped to handle the reported localized inflammation and influx of inflammatory cytokines, leading to their own demise [23]. To fully understand the inflammatory profile of these patients, it is necessary to assess remaining key cytokines involved in the destruction of beta cells. Furthermore, this is a relatively small study of pancreas and serum samples from T1D patients and healthy controls; hence analyzing more patients is required to develop a more concise picture of the mediators and key players in pancreatic β -cell destruction and T1D.

CONCLUSION

The intricate relationship between IL-8 and MPO in the context of T1D offers a compelling avenue for unraveling the heterogeneity within this autoimmune disease. The journey to dissect this multifaceted autoimmune disorder is a promising frontier, and the interplay between IL-8 and MPO may hold the key to a more nuanced and tailored approach to managing this complex autoimmune disease.

The results of the present study show that IL-8 and IL-8 receptor expression are elevated in the pancreases of people diagnosed with recent onset T1D with circulating IL-8 and the leukocyte activation marker MPO also raised. Furthermore, this study demonstrated a clear trend for increased HbA1c levels and IL-8 in patients with high IL-8 levels. However, to fully understand the inflammatory roles of IL-8 and MPO in the process of beta cells destruction, further research is needed, to develop a more concise picture of these cytokine mediators in the process of pancreatic beta cell destruction in T1D.

Understanding the synergistic effects of IL-8 and MPO in T1D pathogenesis opens avenues for targeted therapeutic interventions. Modulating IL-8 and MPO levels, either individually or in combination, could represent a novel strategy to disrupt the pro-inflammatory and oxidative processes that drive beta cell destruction.

Table 1. Demographic characteristics of N=42 recent onset T1D based on IL-8 levels $\leq$ or $>$ 62 pg/mL (the cut-off for high IL-8 levels). n= 10 T1D patients had an IL-8 level $\leq$ 62 pg/mL and n=32 had an IL-8 level $>$ 62 pg/mL. *P-values were calculated via a Student's T-test or Mann-Whitney test where appropriate. IL-8; interleukin 8, BMI; body mass index, HbA1c; glycated haemoglobin, TNF α ; tumour necrosis factor alpha, MPO; myeloperoxidase. *TNF α levels were not available for all patients. IL-8 cut-off was calculated via the average IL-8 of controls $\pm$ 2 S.Ds, crossed reference with Zhang et al., 2017 [7].*

N=42	<i>Patients with Recent Onset Type 1 Diabetes</i>		
IL-8 levels (pg/mL)	$\leq$ 62 pg/mL	$>$ 62 pg/mL	P-value
Total (n)	10	32	
Age years (mean $\pm$ S.D)	17.20 $\pm$ 6.80	15.91 $\pm$ 7.86	0.6424
Gender male n (%)	7 (70%)	11 (34%)	0.5473
BMI kg/m ² (median [IQR])	21.43 (18.10-25.00)	18.00 (15.13-20.92)	0.0583
HbA1c % (mean $\pm$ S.D)	8.95 $\pm$ 1.91	11.28 $\pm$ 2.70	0.0154
Blood glucose (mean $\pm$ S.D)	383.0 $\pm$ 137.4	258.8 $\pm$ 67.55	0.0151
C-peptide ng/mL (mean $\pm$ S.D)	0.75 $\pm$ 0.30	0.74 $\pm$ 0.37	0.988
TNF α pg/mL (mean $\pm$ S.D) *	0.69 $\pm$ 0.06	2.2 $\pm$ 4.10	0.034
MPO pg/mL (mean $\pm$ S.D)	13.2 $\pm$ 10.6 *10 ⁴	13.5 $\pm$ 11.3 *10 ⁴	0.9282

Figure 1. Expression of IL-8 (CXCL8) and its receptor CXCR1 in pancreas samples from Type 1 diabetes cases and non-diabetic controls. Expression levels in archived pancreas samples from 29 type 1 diabetes cases and 7 controls were determined by hybridisation with gene specific probes using the nanoString nCounter system. Significance of differences in expression was evaluated by Mann-Whitney test. * represents $p < 0.05$. Bars represent median with 95% confidence intervals.

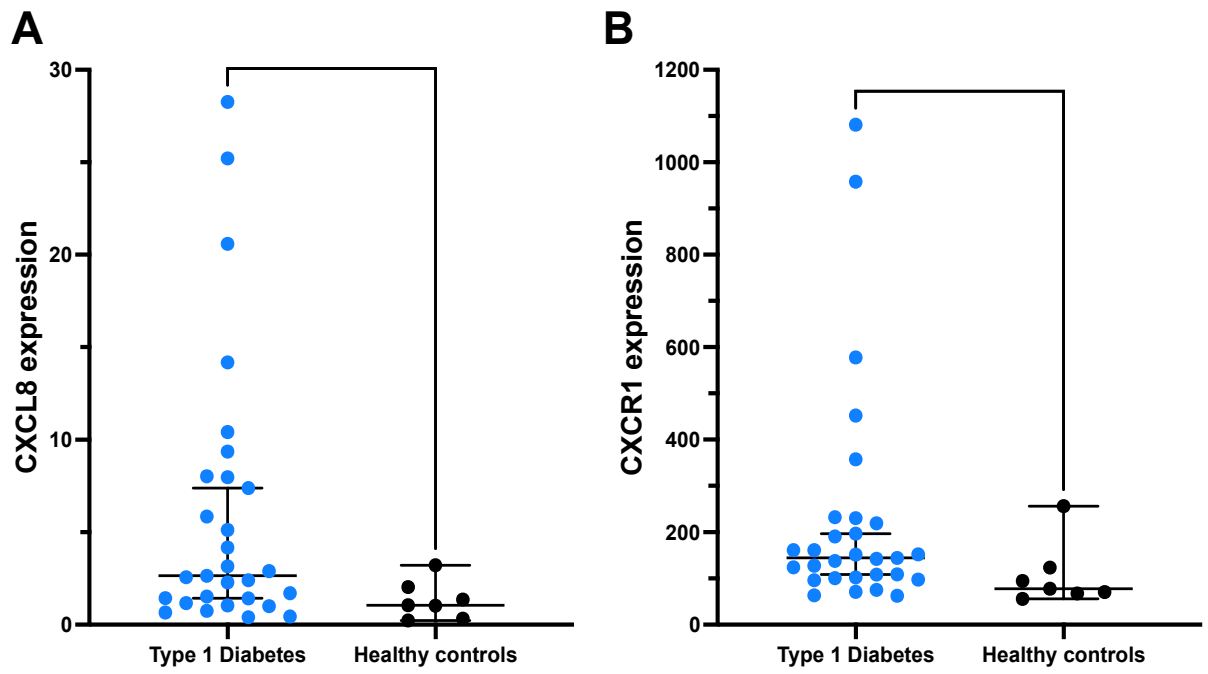


Figure 2. Assessment of cytokine levels in patients with recent onset T1D compared with healthy controls. The figure shows the comparison of IL-8 levels (A) and MPO (B) in N=42 patients with recent onset T1D against N=11 healthy controls. *P*-values were calculated using a student's *T*-test or Mann-Whitney test where appropriate. *P*-value *** < 0.002 , * < 0.0349 , bars represent standard error.

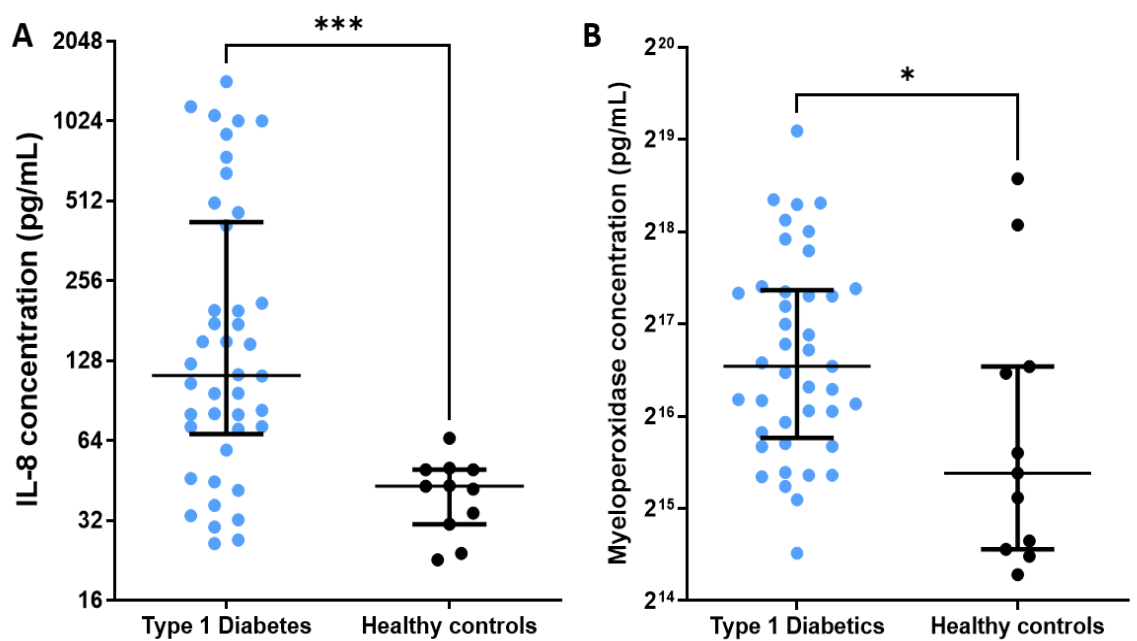


Figure 3. Correlation of IL-8 and MPO levels in recent onset T1D (A) and Healthy controls (B). The figure shows a significant positive correlation of IL-8 and MPO levels in recent onset T1D patients ($r=0.312$, $p\text{-value}=0.024$). However, there appeared to be no significant correlation between IL-8 and MPO in healthy controls ($r=-0.273$, $p\text{-value}=0.418$). Correlation coefficient (r) and p -values were calculated using Spearman's correlation coefficient.

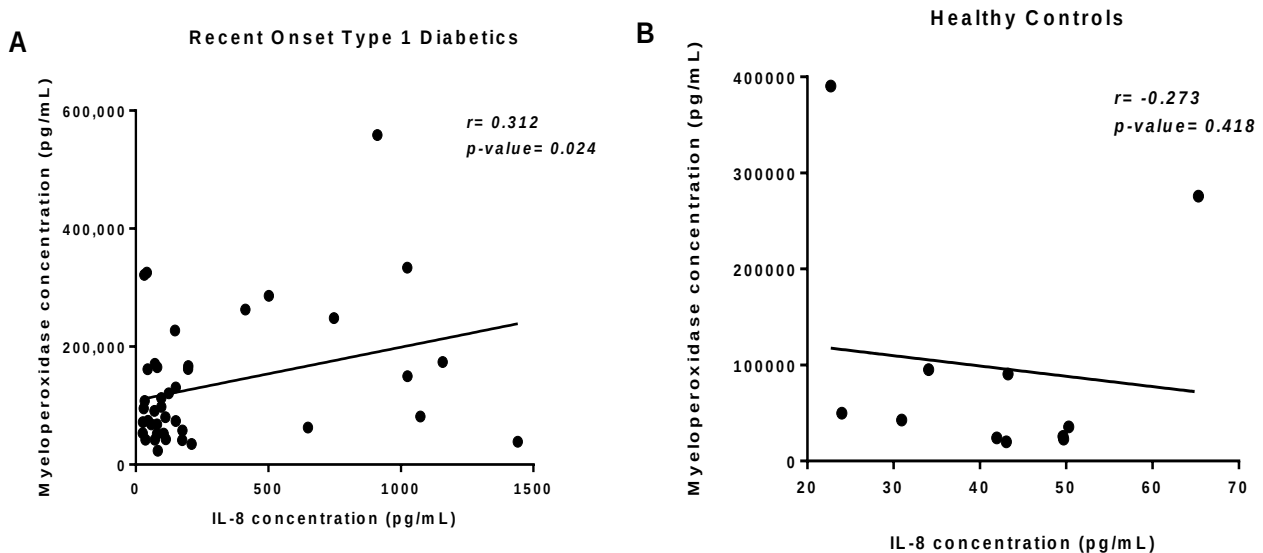


Figure 4. Shows a significant increase in HbA1c (A) (mean $\pm$ S.D, 11.28 ± 2.7 vs 8.9 ± 1.91 , $p\text{-value}=0.0154$) and blood glucose (B) (mean $\pm$ S.D, 383.0 ± 137.4 vs 258.8 ± 67.55 , $p\text{-value}=0.0151$) levels in patients with T1D based on high or low IL-8 levels.

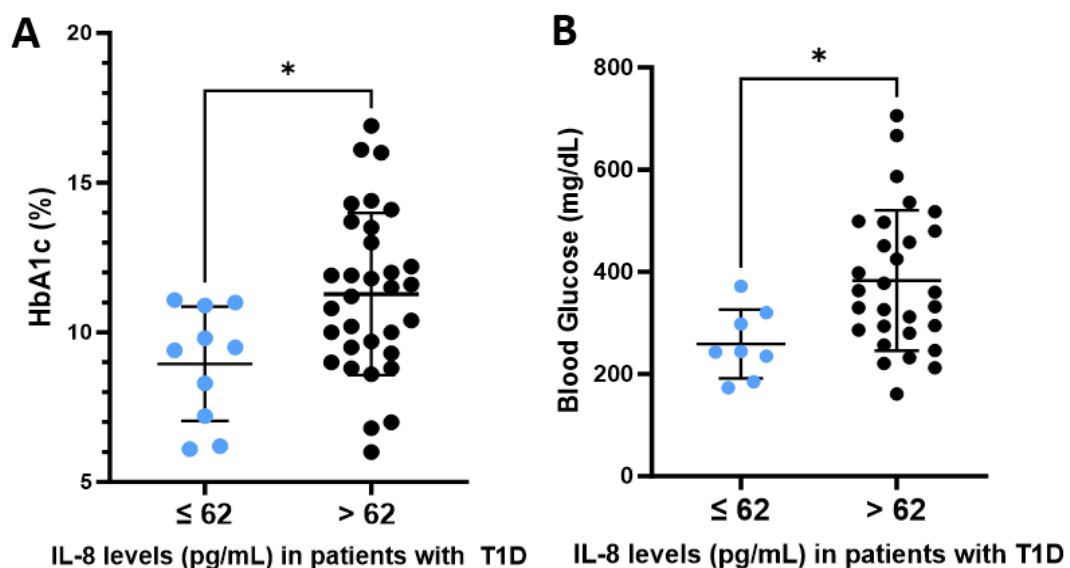
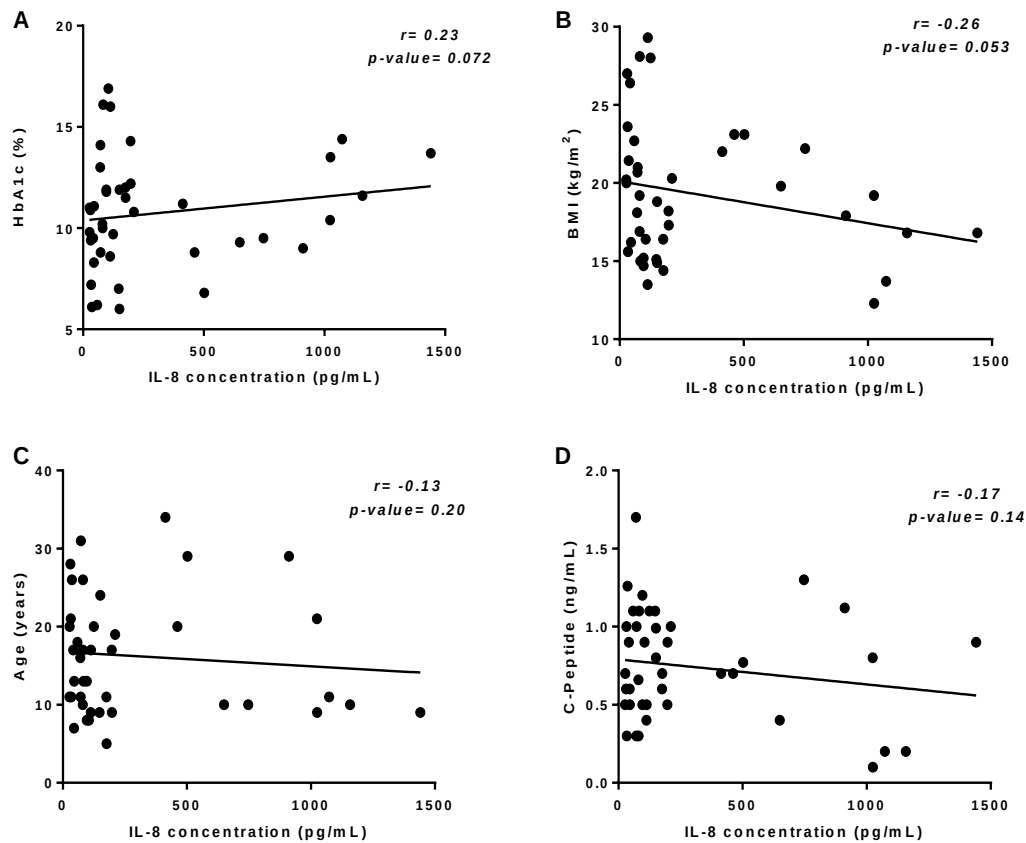


Figure 5. Correlation of IL-8 levels in N=42 recent onset T1D with demographic data. The results showed a positive correlation with HbA1c (A) ($r=0.23$, $p\text{-value}=0.072$) and BMI (B) ($r=-0.26$, $p\text{-value}=0.053$) against IL-8 levels, however these were not statistically significant. There appeared to be no significant correlation with age (C) ($r=-0.13$, $p\text{-value}=0.20$) and C-peptide (D) ($r=-0.17$, $p\text{-value}=0.14$) against IL-8 levels. *Correlation coefficient (r) and p -values were calculated using a Pearson's correlation or a Spearman's correlation where appropriate.*



REFERENCES

1. Bluestone, J.A., K. Herold, and G. Eisenbarth, *Genetics, pathogenesis and clinical interventions in type 1 diabetes*. Nature, 2010. **464**(7293): p. 1293-300.
2. Lu, J., et al., *Cytokines in type 1 diabetes: mechanisms of action and immunotherapeutic targets*. Clin Transl Immunology, 2020. **9**(3): p. e1122.
3. Nepom, G.T., M. Ehlers, and T. Mandrup-Poulsen, *Anti-cytokine therapies in T1D: Concepts and strategies*. Clin Immunol, 2013. **149**(3): p. 279-85.
4. Herold, K.C., et al., *An Anti-CD3 Antibody, Teplizumab, in Relatives at Risk for Type 1 Diabetes*. N Engl J Med, 2019. **381**(7): p. 603-613.
5. Citro, A., et al., *Innate Immunity Mediated Inflammation and Beta Cell Function: Neighbors or Enemies?* Front Endocrinol (Lausanne), 2020. **11**: p. 606332.
6. Cesta, M.C., et al., *The Role of Interleukin-8 in Lung Inflammation and Injury: Implications for the Management of COVID-19 and Hyperinflammatory Acute Respiratory Distress Syndrome*. Front Pharmacol, 2021. **12**: p. 808797.
7. Zhang, J. and C. Bai, *Elevated Serum Interleukin-8 Level as a Preferable Biomarker for Identifying Uncontrolled Asthma and Glucocorticosteroid Responsiveness*. Tanaffos, 2017. **16**(4): p. 260-269.
8. Zozulinska, D., et al., *Serum interleukin-8 level is increased in diabetic patients*. Diabetologia, 1999. **42**(1): p. 117-8.
9. Lo, H.C., S.C. Lin, and Y.M. Wang, *The relationship among serum cytokines, chemokine, nitric oxide, and leptin in children with type 1 diabetes mellitus*. Clin Biochem, 2004. **37**(8): p. 666-72.
10. Pan, X., et al., *Chemokines in Type 1 Diabetes Mellitus*. Front Immunol, 2021. **12**: p. 690082.

11. Van Sickle, B.J., et al., *Increased circulating IL-8 is associated with reduced IGF-1 and related to poor metabolic control in adolescents with type 1 diabetes mellitus*. Cytokine, 2009. **48**(3): p. 290-4.
12. Chettab, K., et al., *Acute hyperglycaemia induces changes in the transcription levels of 4 major genes in human endothelial cells: macroarrays-based expression analysis*. Thromb Haemost, 2002. **87**(1): p. 141-8.
13. Torabi, F., et al. *Differential expression of genes controlling lymphocyte differentiation and migration in two distinct endotypes of type 1 diabetes*. Diabet Med. 2023 Sep;**40**(9):e15155..
14. Devaraj, S., M.R. Dasu, and I. Jialal, *Diabetes is a proinflammatory state: a translational perspective*. Expert Rev Endocrinol Metab, 2010. **5**(1): p. 19-28.
15. Dakovic, D., et al., *Salivary interleukin-8 levels in children suffering from type 1 diabetes mellitus*. J Clin Pediatr Dent, 2013. **37**(4): p. 377-80.
16. Donath, M.Y., C.A. Dinarello, and T. Mandrup-Poulsen, *Targeting innate immune mediators in type 1 and type 2 diabetes*. Nat Rev Immunol, 2019. **19**(12): p. 734-746.
17. Aratani, Y., *Myeloperoxidase: Its role for host defense, inflammation, and neutrophil function*. Arch Biochem Biophys, 2018. **640**: p. 47-52.
18. Gustavsson, C.G. and C.D. Agardh, *Markers of inflammation in patients with coronary artery disease are also associated with glycosylated haemoglobin A1c within the normal range*. Eur Heart J, 2004. **25**(23): p. 2120-4.
19. Liu, S., et al., *Association between Inflammation and Biological Variation in Hemoglobin A1c in U.S. Nondiabetic Adults*. J Clin Endocrinol Metab, 2015. **100**(6): p. 2364-71.
20. Stoica, R.A., et al., *Interleukin-8, CXCL10, CXCL11 and their role in insulin resistance in adult females with subclinical hypothyroidism and prediabetes*. J Clin Transl Endocrinol, 2022. **28**: p. 100299.

21. Redondo, M.J., et al., *Serum adiposity-induced biomarkers in obese and lean children with recently diagnosed autoimmune type 1 diabetes*. *Pediatr Diabetes*, 2014. **15**(8): p. 543-9.
22. Ross, C., et al., *The Prevalence of Islet Autoantibodies in Children and Adolescents With Type 1 Diabetes Mellitus: A Global Scoping Review*. *Front Endocrinol (Lausanne)*, 2022. **13**: p. 815703.
23. Roep, B.O., et al., *Type 1 diabetes mellitus as a disease of the beta-cell (do not blame the immune system?)*. *Nat Rev Endocrinol*, 2021. **17**(3): p. 150-161.