Corneal Langerhans cells in children with celiac disease

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Celiac disease (CeD) is a common small bowel enteropathy characterized by an altered adaptive immune system and increased mucosal antigen presenting cells. This study aims to establish if quantification of corneal Langerhans cells (LCs) using corneal confocal microscopy (CCM) could act as a surrogate marker for antigen presenting cell status and hence disease activity in children with CeD. Twenty children with stable CeD and 20 age-matched controls underwent CCM and quantification of central corneal total, mature and immature LC density. There was no difference in age (11.78 ± 1.7 vs. 12.83 ± 1.9; \( P = 0.077 \)) or height (1.38 ± 0.14 vs. 1.44 ± 0.13; \( P = 0.125 \)). BMI (18.81 ± 3.90 vs. 22.26 ± 5.47; \( P = 0.031 \)) and 25 OHD levels (43.50 ± 13.36 vs. 59.77 ± 22.45; \( P = 0.014 \)) were significantly lower in children with CeD compared to controls. The total (33.33(16.67–59.37) vs. 51.56(30.21–85.42); \( P = 0.343 \)), immature (33.33(16.67–52.08) vs. 44.79(29.17–82.29); \( P = 0.752 \)) and mature (1.56(0–5) vs. 1.56(1.04–8.33); \( P = 0.752 \)) LC density did not differ between the CeD and control groups. However, immature (\( r = 0.535, P = 0.015 \)), mature (\( r = 0.464, P = 0.039 \)) and total (\( r = 0.548, P = 0.012 \)) LC density correlated with age. Immature (\( r = 0.602, P = 0.038 \)) and total (\( r = 0.637, P = 0.026 \)) LC density also correlated with tissue transglutaminase antibody (Anti-TtG) levels assessed in 12/20 subjects with CeD. There was no difference in corneal LC density between children with CeD and controls. However, the correlation between corneal LC density and anti-TtG levels suggests a relationship with disease activity in CeD and requires further study.

Celiac disease (CeD) affects ~ 0.7% of the world population1, but may be more prevalent in the Middle East2,3, especially in Qatar4. It is characterized by varying degrees of intestinal malabsorption, caused by an inappropriate immune response to ingested wheat gluten containing gliadin. Histopathological studies demonstrate villous atrophy with defective transepithelial5,6 and paracellular uptake of gliadin by the intestinal mucosa of patients with active celiac disease.

Circulating dendritic cells are recruited to the inflamed mucosa in those with active CeD, and indeed, there is a significant increase in the number of dendritic cells (DC) in the lamina propria of patients with active celiac disease, which reverts to normal with a gluten-free diet7,8. DCs isolated from patients with active celiac disease behave as APCs and transcribe IFN-gamma9, a key cytokine in the pathogenesis of CeD. The level of auto-antibodies to the enzyme transglutaminase 2 (TG2) and gliadin in gluten-consuming subjects are used as a diagnostic adjunct and marker of disease activity. Intriguingly, TG2 is expressed on most cell surfaces including monocytes and APCs, suggesting that it may facilitate the uptake of gluten10.

Corneal Langerhans cells (LC’s) are APCs which modulate the immune response in the cornea11,12. We have used corneal confocal microscopy (CCM) a rapid, non-invasive and well-tolerated ophthalmic imaging technique to quantify the number of mature and immature corneal LC’s11–15. Moreover, we and others have shown increased LC’s in patients with type 1 diabetes11,16, latent autoimmune diabetes of adults (LADA)16, multiple sclerosis (MS)12, long-COVID15, dry eye disease16, systemic lupus erythematosus (SLE)19, fibromyalgia18, thyroid-associated ophthalmopathy21, and chronic inflammatory demyelinating polyneuropathy (CIDP)22. These studies suggest that corneal immune cells are associated with a number of immune and inflammatory diseases.

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In the present study we have quantified mature, immature and total numbers of LCs and related them to clinical parameters and anti-TtG level in children with CeD.

**Results**

There was no significant difference in age, height, or tissue transglutaminase antibody between children with CeD and the control group. BMI and vitamin D were significantly lower in children with CeD compared to the control group (Table 1).

**Langerhans cells.** The total, immature and mature LC density in the central cornea did not differ between children with CeD and controls (Fig. 1a,b) (Table 2).

| Age                  | Controls (n=20) | CeD (n=20) | P-value |
|----------------------|----------------|------------|---------|
| Duration of disease  |                |            |         |
| Height (m)           | 1.44±0.13      | 1.38±0.14  | 0.125   |
| Body Mass Index (kg/m²) | 22.26±5.47    | 18.81±3.90 | 0.031   |
| Tissue Transglutaminase antibody (U/mL) | 0–3 | 12.90(1–165)* | - |
| Hemoglobin (g/dl)    | 125.50±7.84    | 125.63±7.45| 0.963   |
| Platelets x 10⁹/L    | 333.75±73.20   | 325.42±77.51| 0.768   |
| WBC x 10⁹/L          | 7.72±1.98      | 6.73±1.89  | 0.171   |
| 25 OHD (ng/mL)       | 59.77±22.45    | 43.50±13.36| 0.014   |
| Vitamin B₁₂ (ng/mL)  | 180–914        | 321.84±89.40| -         |
| Folic acid (nmol/L)  | 11.3–47.6      | 18.60(12.20–45.50)| -     |
| Serum Iron (μmol/L)  | 13.65±2.76     | 11.46±7.67 | 0.709   |

**Table 1.** Clinical demographic and laboratory measures in control subjects and children with CeD. Data is presented as mean ± SD or median (range), Anti TtG, vitamin B12 and folic acid levels were compared with the normal laboratory range. CeD: Celiac Disease, BMI: Body Mass Index, WBC: White blood Cell Count. *Anti-TtG was available in 12/20 children with CD. Significant differences between groups are highlighted in bold.

**Figure 1.** (a) A CCM image of the sub-basal nerve plexus in a healthy control and (b) a child with celiac disease (CeD) with mature (red arrow) and immature LCs (green arrow).

| Density (no./mm²)         | Controls       | Celiac Disease | P-value |
|---------------------------|----------------|----------------|---------|
| Immature LC               | 44.79(29.17–82.29) | 33.33(16.67–52.08) | 0.752   |
| Mature LC                 | 1.56(1.04–8.33)  | 1.56(0–5)      | 0.752   |
| Total LC                  | 51.56(30.21–85.42)| 33.33(16.67–59.37)| 0.343   |

**Table 2.** Langerhans cells in children with Celiac Disease and controls. *Data is presented as mean ± SD, CeD: celiac disease, LC: Langerhans cells.
Methods

Children with a confirmed diagnosis of celiac disease and a positive serology test for anti-TtG antibodies (available in 12/20) with a disease duration of 4.49 ± 4.02 years and 20 healthy controls were recruited. Inclusion criteria were age between 8 and 17 years and a diagnosis of CeD. Exclusion criteria were any history of a cause of neuropathy, malignancy, vitamin B12 or folate deficiency, liver, or renal dysfunction. Participants were also excluded in they had corneal pathology, allergy to the eye-drops or previous ocular trauma or surgery in the past six months. Participants with a history of or current contact lens use were excluded. The study was approved by the Ethics Committee of Weill Cornell Medicine-Qatar (IRB 1700032) and Sidra Medicine (IRB 1500758–3) and was undertaken according to the principles of the Helsinki Declaration. Written informed consent and assent were obtained from all participants and their parents.

Corneal confocal microscopy procedure. Corneal confocal microscopy was undertaken using the Heidelberg Retina Tomograph Cornea Module (Heidelberg Engineering, Germany). Anesthetic drops Bausch & Lomb Minims® (Oxybuprocaine hydrochloride 0.4% w/v) were used to numb both eyes to limit irritation and discomfort during the examination. A drop of hypotears gel (Carbomer 0.2% eye gel) was placed on the tip of
the objective lens and a sterile disposable TomoCap was placed over the lens, allowing optical coupling of the objective lens to the cornea. Images were captured from the central cornea to quantify corneal Langerhans cells in the sub-basal layer. The investigator (HG) was blind to the study group when performing CCM and analyzing CCM images.

**Image selection and quantification.** Six images were selected from the central cornea, excluding those with pressure lines and out of focus images. Langerhans cells were identified as white, bright structures. Total, mature (with dendrites) and immature (without dendrites and a total end-to-end length less than 25 µm) L.C.s were counted manually using CCMetrics software. The investigator was blind to disease and control group during selection and quantification of the CCM images using anonymized codes for each participant.

**Statistical analysis.** All statistical analyses were performed using IBM SPSS Statistics software Version 27 and P < 0.05 was considered statistically significant. Normality of the data was assessed using the Shapiro–Wilk test, histograms and normal Q-Q plot. Data are expressed as mean ± SD for the normally distributed variables and as median(range) for the skewed variables. Inferential analyses were conducted for the corneal nerve parameters and clinical demographics using both parametric (T-test) and non-parametric (Mann–Whitney U) tests, with post-hoc adjustment. To investigate the association between corneal nerve metrics and clinical variables, Pearson and Spearman correlation were performed as appropriate. Grayphad prism version 9 was used to build dot plots.

**Data availability**

Data is available upon reasonable request to the corresponding author.

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**Author contributions**

R.A.M. and A.K.A. were the guarantors and designed the study; H.G., I.M., I.N.P., G.P., A.K., S.S., B.A., M.E., W.A., H.A., P.S., S.A. H.A., K.H., M.A.H., F.A., and K.A. participated in the data acquisition; H.G. and M.F. undertook the image analysis. H.G. undertook data analysis, data interpretation and drafted the initial manuscript; R.A.M., A.K.A., and K.H. revised the article critically for important intellectual content.

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**Competing interests**

The authors declare no competing interests.

**Additional information**

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