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LC/DAD analysis of serum biogenic amines in patients with diabetes mellitus, chronic urticaria and Hashimoto's thyroiditis

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Purpose/Objective: The biogenic amines putrescine (Put), histamine (His), spermidine (Spd), *N*-acetyl putrescine (NAP), *N*-acetyl spermidine (NAS), dopamine (Dop), epinephrine (Epi), norepinephrine (NE) and spermine (Spm) are a group of naturally occurring compounds exerting a large number of biological effects.

This study was commenced to elucidate the role of biogenic amines as possible diagnostic markers for three autoimmune diseases: *Diabetes mellitus*, *Chronic urticaria*, and *Hashimoto's thyroiditis*.

Materials and methods: This study involved 20 patients with *Diabetes mellitus*, 20 patients with *Chronic urticaria*, eight patients with Hashimoto's thyroiditis, and 20 healthy volunteers. We precipitated serum proteins using 0.4 M $HClO_4$. At pH 8.0 we performed derivatization with dansyl-chloride. 50 μl of prepared serum samples were injected into LC/DAD, in conditions of gradient elution, on C18 column. Commercially available Put, His, Spd, NAP, NAS, Dop, Epi, NE, and Spm were dissolved in different concentrations in ultra pure water; treated in the same way as serum samples and injected into LC. Calibration curves were made by plotting peak area values against the respective concentrations of standards. The qualitative analysis was done using the method of retention time, and quantitative analysis using external calibration. The recovery study was carried out using real serum sample from healthy control, by spiking techniques.

Results: Retention times were 6.6 min for NAS, 8.8 min for NAP, 9.1 min for Put, 10.1 min for His, 13.2 min for Spd, 13.9 min for NE, 14.7 min for Epi, 14.9 min for Dop, and 15.4 min for Spm, respectively. Obtained data showed excellent linearity of calibration curves for Put, His, Spd, NAP, NAS, Dop, Epi, NE, and Spm. Compared to controls, His levels in *Diabetes mellitus* patients were statistically higher; in *Chronic urticaria* patients levels of Put and His were lower; Spd, NE and Epi levels were enhanced; Put was statistically lower and Spd higher in *Hashimoto's thyroiditis* patients. *Chronic urticaria* patients were the only in whose serum NE was found. NAP, NAS, Dop, and Spm were under limits of detection.

Conclusions: Preliminary results from this study showed diverse distribution of investigated biogenic amines indicating different activation of metabolic pathways controlling biogenic amine biosynthesis and degradations in analyzed autoimmune diseases.

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Levels of inflammatory mediators in gingival crevicular fluid from patients with periodontal disease before and after treatment

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Purpose/Objective: The purpose of the present research was to determine the levels of IL-1α, IL-1β, TNF-α, MMP-3 and MMP-8 in gingival crevicular fluid (GCF) of subjects with chronic periodontitis before and after non surgical treatment.

Materials and methods: Clinical measurements were carried out in 11 patients diagnosed with chronic periodontitis and 11 periodontally healthy controls. The clinical indexes evaluated were: gingival index (GI), plaque index (PI), bleeding on probing (BOP), probing depth (PD) and attachment loss (AL). The measurements were taken at six sites per tooth in all teeth in each subject. GCF samples were taken from one tooth per quadrant, and the levels of these mediators were measured using an ELISA test.

Results: A statistically significant difference ($P < 0.05$) was observed in all the clinical parameters between patients and control group before and after periodontal treatment. Correlations between levels of mediators with the clinical parameters were not observed. Statistically significant differences were found between patients and control group in relation with levels of inflammatory mediators ($P < 0.05$) before and after treatment. The levels of IL-1α, IL1-β, TNF-α, MMP-3 and MMP-8 decreased in 44.2%, 48.35%, 53.28%, 62.16% y 34.03% respectively.

Conclusions: Periodontal therapy reduced the levels of the inflammatory mediators evaluated in this study, which were significantly associated with the severity of periodontal disease. This research was supported by CDCH PG: 10-00-7070-2007.

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Measuring autoantibodies against IL-17F and IL-22 in autoimmune polyedocrine sydnrome type I

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Purpose/Objective: Patients with autoimmune polyendocrine syndrome type I (APS I) have at least two of the three disease components adrenal insufficiency, hypoparathyroidism and chronic mucocutaneous candidiasis. Various other organ-specific autoimmune manifestations are common, as well as a number of ectodermal symptoms. The underlying cause of APS I is mutations in the gene encoding the autoimmune regulator protein AIRE. Deficiency of this protein leads to loss of immunologic tolerance and release of autoreactive T-cells from thymus into the periphery.

Patients frequently develop high titers of autoantibodies against molecular targets in their affected organs. The pathological role of these antibodies is unknown, but they have become important markers for APS I and other autoimmune conditions. Autoantibodies against interleukin (IL) -17A, IL-17F and IL-22 have recently been described in patients with APS I, and their presence is reported to be highly correlated to chronic mucocutaneous candidiasis (CMC). The aim of this study was to develop a robust high-throughput radioligand binding assays (RLBA) measuring IL-17F and IL-22 antibodies, and to compare them with current enzyme-linked immunosorbent assays (ELISA) of IL-17F and IL-22; moreover to correlate the presence of these antibodies to the presence of CMC.

Materials and methods: A total of five RLBAs were developed based on IL-17F and IL-22 monomers and homo- or hetero dimers. As these