Granulomatous Diseases of the Oral Cavity: A Proteomic Approach

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Introduction

Orofacial granulomatosis (OFG) is a chronic granulomatous inflammatory disease that presents with relapsing and remitting lip swelling, cobblestone inflammation of the mucosa and oral ulceration (1).

OFG may be a precursor to gut Crohn's Disease (CD), particularly in the paediatric population. (2) Worryingly the incidence of both OFG and CD has been on the rise in Western countries. (3-4)

However, not all OFG patients have, or ever develop CD. Therefore, whether OFG and oral CD (OCD) are one and the same disease is currently a matter of debate. (5) Unlike gut CD research into the aetiology and pathogenesis of OFG and oral CD is severely lacking.

A proteomic approach can provide invaluable information with a focus on phenotypic differences between OFG and OCD. The proximity extension assay (PEA) is a high throughput homogenous multiplex immunoassay which is both sensitive and highly specific. (6)

Saliva offers specific advantages as a diagnostic marker for oral inflammatory diseases as the salivary proteome is representative of the immunopathogenic events occurring in mucosal tissues. (7)

Aims

Utilise the PEA to determine the levels of 188 different immunoregulatory proteins in the saliva of patients with OFG/OCD, Oral Lichen Planus (an unrelated oral inflammatory condition) and healthy volunteers.

Analyse data to determine proteomic differences between OFG and OCD thus establishing if they are separate entities or the same disease process.

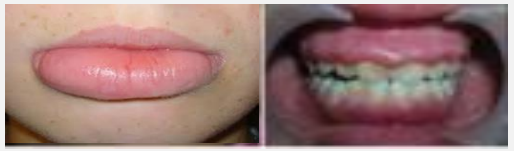

Figure 1 – Presenting symptoms of Orofacial granulomatosis A – labial swelling. B – gingival hyperplasia.

Study Methods

In accordance with West of Scotland Ethics (16/WS/0159) we recruited 35 patients with OFG/OCD and 28 patients with Oral Lichen planus from NHS GGC Oral Medicine outpatient clinics and 30 healthy volunteers from University of Glasgow staff and student body.

Salivary sample collection was in line with clinical assessment and routine investigations and samples processed prior to plating for PEA.

Demographics recorded at first visit included gender, age, medical history, smoking status and disease activity scores.

Immune and inflammatory biomarker analysis was performed using PEA. (Olink, Uppsala, Sweden)

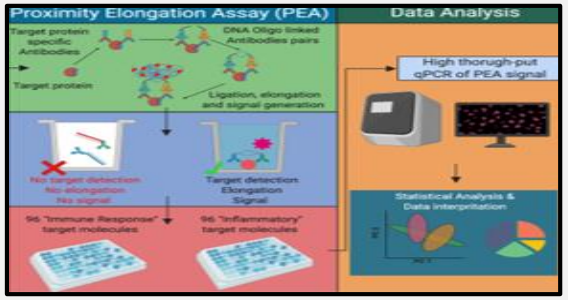

Figure 2 – Schematic diagram of salivary proteome analysis by PEA assay.

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Results

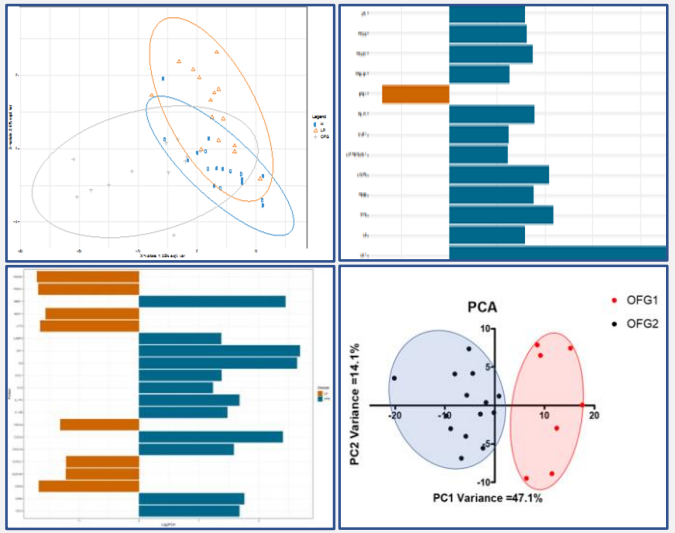

Figure 3: PCA to demonstrate distinct profile of salivary proteome between health, OLP and OCD/OFG (A) Upregulation of 12 proteins and downregulation of 1 protein in OFG/OCD as compared to Health (B) Upregulation of 12 proteins and downregulation of 8 proteins in OFG/OCD as compared to OLP © PCA to demonstrate two distinct clusters in OFG/OCD group

Discussion:

PCA utilising immune and inflammatory protein PEA plates highlighted a distinct profile of the salivary proteome of OFG/OCD patients when compared to that of healthy volunteers and OLP patients. (Figure 3A and 3B).

Interestingly the salivary proteome of OLP, itself a chronic inflammatory disease of the oral cavity, differs significantly from the profile of OFG/OCD (Figure 3C) but not from the profile generated from healthy volunteers.

When focusing on the OFG/OCD group PCA demonstrates two separate clusters (Figure 3D) which broadly align to the presence or absence of gut symptoms thus adding evidence to the argument that OCD and OFG are separate entities requiring different management strategies.