PRELIMINARY STUDY ON SALIVA FROM DIABETIC AND NON-DIABETIC INDIVIDUALS BY POLYACRYLAMIDE GEL ELECTROPHORESIS

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ABSTRACTS

Human saliva contains a large number of proteins which can be separated using polyacrylamide gel electrophoresis (PAGE). In this study the protein profiles of whole saliva of diabetic and non-diabetic were compared. Considerable variations between individuals in the protein profiles were observed. The saliva from diabetic patients appeared to have more of proline-rich protein bands in the molecular weight region below 56 KDa. Further investigations using individual gland saliva should be carried out.

KEYWORDS

Whole saliva; diabetics; non-diabetics; Polyacrylamide gel electrophoresis (PAGE); proline-rich proteins

INTRODUCTION

Human saliva is secreted by three pairs of major glands (parotid, submandibular and sublingual) and numerous minor ones (labial, buccal, palatine and glossopalatine). The submandibular and sublingual are the major contributors of the resting saliva and the saliva secreted by these glands are more viscous in nature compared to the stimulated saliva. The parotid glands are the main contributors of stimulated saliva. The minor glands secretions function mainly as moisturizer. The secretions of both major and minor glands together with gingival exudate(crevicular fluid) micro-organisms and cell debris form the mixed or whole saliva (oral fluid).

There are a large number of proteins in human saliva with the concentration ranging from ~0.50 to 3.0 mg/ml (1). α-Amylase and proline-rich proteins which comprising ~25% and ~65% respectively, are the major proteins of glandular origin. The remaining ~10% consists of other proteins which include lactoferrin, histatins, cystatins and immunoglobulins. Most of the proteins are salivary gland specific (e.g. (-amylase, proline-rich proteins, histatins) and are synthesised within the salivary glands but some, such as albumin, are derived from serum (1).

The use of saliva for clinical and diagnostic purposes has opened up a new era in salivary analysis. It has been demonstrated that the composition and flow rate of saliva are altered in several systemic diseases such as Sjögren's syndromes and cystic fibrosis (2). Essential hypertension (3), diabetes mellitus (4), sarcoidosis and inflammatory disease of the salivary glands (5) are known to alter the protein composition of saliva.

Electrophoretic methods such as sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) has been used in the analysis of saliva (2,6). The salivary analysis, as compared to blood analysis, is more attractive because it offers a simple rapid non-invasive method which is suitable for short- and long-term monitoring of pathological disorders and drug therapy. Finestone *et al.*(6) has reported that PAGE of parotid saliva using Buffalo Black staining showed that the γ-globulin level of the diabetic patients is higher than that of the non-diabetic subjects, as indicated by diffuse bands in the γ-globulin region of the saliva samples from diabetic patients. In our study, the protein in whole saliva of non-diabetic subjects and diabetic patients separated on SDS-PAGE using Coomassie-blue R250 staining were studied and compared.

MATERIALS AND METHODS

Materials

Acrylamide, bovine serum albumin standard, N,N'-methylene bisacrylamide, protein markers, sodium dodecylsulphate, TEMED and Tris were purchased from Bio-Rad (U.S.A.). Coomassie Brilliant Blue R-250, glycine and glycerol were supplied by BDH Chemicals Ltd. (England), β-mercaptoethanol was purchased from Serva (U.S.A.), bromophenol blue was supplied by Koch-light Lab. Ltd. (England), acetic acid was from R&M Marketing (U.K.) and ammonium persulphate was purchased from Merck (Germany).

Methods

(a) Saliva collection

Saliva samples were donated by diabetes mellitus patients treated at the University Hospital, Kuala Lumpur. Normal or non-diabetic saliva samples were obtained from donors who had no history of diabetes mellitus. Saliva samples were collected by expectoration. The samples were refrigerated within 1 hour of collection, then centrifuged for 20 min. at 5000 x g and 4 (C. The clear supernatant was collected and concentrated using speed-vacuum centrifugation. These samples were then estimated for protein content and then used for SDS-PAGE.

(b) Estimation of protein content

Protein content of the concentrated saliva samples was determined by using Bradford's method (7), with bovine serum albumin as the standard.

(c) SDS-PAGE

SDS-PAGE was carried out according to the method of Laemli *et al.* (8). A 12% separating gel and 4% stacking gel

were used. About 15 µg of the concentrated saliva samples was loaded into the wells of the stacking gel separately. Protein markers with known molecular weights ranging from 14.4 KDa to 97.4 KDa for low molecular weights and from 45 KDa to 200 KDa for high molecular weights were loaded at the same time as reference. The electrophoresis was carried out using the Mini-PROTEAN® II dual slab cell (Bio-Rad Lab., U.S.A.) at 150 V for about 1 hour.

When the run was over, the gels were stained by the conventional Coomassie technique, i.e. 3 hours in 0.1% Coomassie Brilliant Blue R-250, 50% methanol and 10% acetic acid. Destaining was carried out overnight in 10% acetic acid solution.

RESULT AND DISCUSSION

Protein band patterns revealed by SDS-PAGE followed by staining with Coomassie Brilliant Blue R-250 is shown in Figure 1.

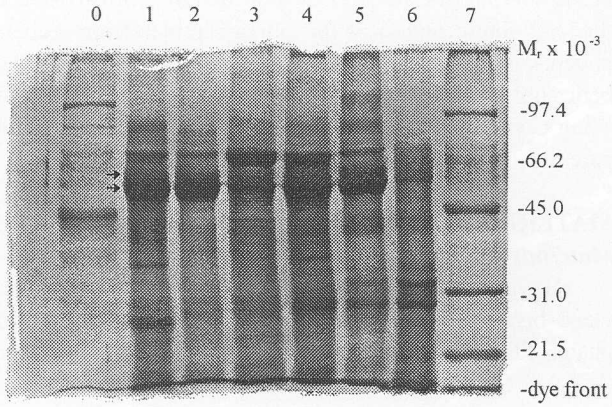

Fig. 1. SDS-PAGE of human whole saliva from diabetic patients (1-4) and non-diabetic subjects (5 & 6) on a 12 % gel. Lanes 0 & 7 are the high- and low-molecular weight protein markers, respectively. The protein load was 15 µg and staining was with Coomassie Blue R-250. The bands indicated by arrows are iso-enzymes of α-amylase.

All the samples showed approximately six blue stained protein bands, two of which corresponded to the glycosylated and non-glycosylated iso-enzymes of α-amylase with molecular weight of 56 KDa and 62 KDa, respectively. The bands with molecular weight of 72 KDa might be that of lactoferrin. Some considerable individual-to-individual variations were observed. Certain bands which were strongly stained in some individuals were either less prominent or not detectable in others.

There are some pink-violet bands detected on the gel. Our study showed that the distribution of these bands is not consistent and some of the samples showed to have more pink-violet bands than the others. The saliva from diabetic patients showed to have more pink-violet bands in the molecular weight region below 56 KDa (Fig 1. Lanes 1-4). The saliva from one of the non-diabetic subjects (Fig. 1. Lane 5) showed that the pink-violet band to be at molecular weight of more than 97.4 KDa. The saliva from another non-diabetic subject did not show this band. These bands were reported to be the proline-rich proteins and stained pink-violet because of the metachromatic effect (9). The metachromatic effect was found to be specific for Coomassie Blue R-250. If the Coomassie Blue G-250 is used, the metachromasia only occurs when the destaining solution is free from organic solvent (9).

SDS-PAGE has been widely used in the study of salivary proteins in human whole saliva. Saliva is heterogenous in nature and its composition is affected by a number of factors. For diagnostic purposes, the time of collection, the type of stimulation used, the oral condition and the medication records of the patients must be specified and taken into consideration. This is to standardise the effect due to circadian rythm on the salivary composition and flowrate.

CONCLUSION

From the SDS-PAGE, saliva from diabetic patients showed to have more of the pink-violet bands or proline-rich proteins in the molecular weight region below 56 KDa. Further investigation using glandular saliva instead of whole saliva and more sensitive methods such as isoelectric focusing should be carried out in order to gain more information.

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