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Changes in Total Protein Level in Human Female Saliva during Ovulatory Functions and Metabolic Disorders

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Abstract- Saliva has been studied extensively as a potential diagnostic tool over the last decade due to its ease and non-invasive accessibility along with its abundance of biomarkers, such as genetic material and proteins. The activity of protein in saliva increased during ovulation. When we monitored salivary protein activity in 40 different women volunteers during various stages of reproduction like (prepubertal, parous, non-parous, menopausal and in the metabolic disorder state diabetic condition it has been observed that highly significant ($p<0.001$) increase in parous ovulatory & non parous ovulatory whereas, highly significant ($p<0.01$)increase was observed in non-parous postovulatory and a highly significant ($p<0.001$) decrease was observed in menopause and diabetic in comparison to prepubertal. A highly significant ($p<0.001$) decrease was observed in menopause in comparison to parous preovulatory, ovulatory, post ovulatory & non-parous ovulatory and postovulatory. A highly significant ($p<0.01$) decrease was observed in diabetic in comparison to menopausal human female subjects. The result revealed that the total protein was considered as testing the saliva instead of blood isa non-invasive loom and it can be used as a biomarker for ovulation detection.

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Changes in Total Protein Level in Human Female Saliva during Ovulatory Functions and Metabolic Disorders

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I. Introduction

As a diagnostic fluids Body fluids like blood, saliva, tears, sweat and urine are a source of pathological biochemical markers in which saliva is an excellent biological fluid that is useful for noninvasive exploration of the human diseases and physiological conditions (Villiger *et al*., 2018). Salivary protein concentration is dependent on gland production at time of day, diet, age, gender and presence of disease (Ferreiro *et al*., 2002). Saliva consists mainly of water, enzymes, ions and amino acids and performs several important functions in oral health. It contains various biomolecules such as proteins, enzymes and hormones. Saliva contains a large number of proteins that participate in the protection of the oral tissues, for instance lysozyme, lactoferrin, lactoperoxidase, immunoglobulins, ag- glutinin and mucins. Nieuw Amerongen and Veer- man 2002, Denny *et al.,* 2008 and Yan *et al.,* 2008 reported about two thousand proteins in human saliva using mass spectrometry. Sanjay *et al.,* 2008 reported significant high total salivary protein in the saliva of cancer patient. In the beginning of menstruation and during ovulation, the protein content of saliva increases considerably, which turns out to be a rich source of nutrient to bacteria, the count of which may increase during menstruation and ovulation. Protein in the serum is made up of albumin and globulin. Albumin is made mainly in the liver which helps tissue growth and healing. Globulins is made up of different proteins called alpha, beta and gamma. Some globulins are made by liver while others are made by immune systems. Salivary proteins, such as mucins, α-amylase, lysozyme, and peroxidase are synthesized and packed into secretory granules in acinar cells. Salivary protein secretion is strongly enhanced by the sympathetic nerve stimulation. Perinpanayagam *et al.,* 1995 reported that small peptide in saliva showed proteolytic activity. Chicharro *et al.,* 1998 indicated that the salivary level of total protein increases also through β –sympathetic activity in salivary glandssince saliva secretion is mainly evoked by the action of androgenic mediators. Hu *et al.,* 2004 and Huang, 2004 studied the structure and function of a large number of proteins in human saliva with traditional biochemical techniques including chromatography, gel electrophoresis, mass spectrometry, immunoassay. Tabak, 2001 and Simpson *et al.,* 2005 also detected various enzymes, enzyme inhibitors, hormones (growth factor) and cytokines (Interlukin-8) in the saliva. Van Nieum Amerongen & Veerman 2002 studied the functional aspect of important proteins in saliva which act as mineralization of the enamel, taste, perception, digestion, inhibition, cell proliferation, chemotaxis, and cell motility. Zachariasen, 1992 also indicated that changes in ovarian hormone levels during puberty, parous pregnancy menstrual cycle and after oral contraceptive use appear to co-relate with decrease in various proteins. Ben-Aryeh *et al.,* 1986 and Arranz *et al.,*1992 also reported increase in total protein and reduction in amylase activity with age in parotid saliva. Lakshmi *et al*., 2015 showed a higher level of salivary total proteins and α-amylase in patients with DM. Panchbhai *et al*., 2010 studied on salivary total proteins, and α-amylase of well-controlled and poorly-controlled DM patients

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compared with healthy individuals and showed a significantly lower level of salivary α-amylase in patients with well-controlled DM compared to healthy subjects.

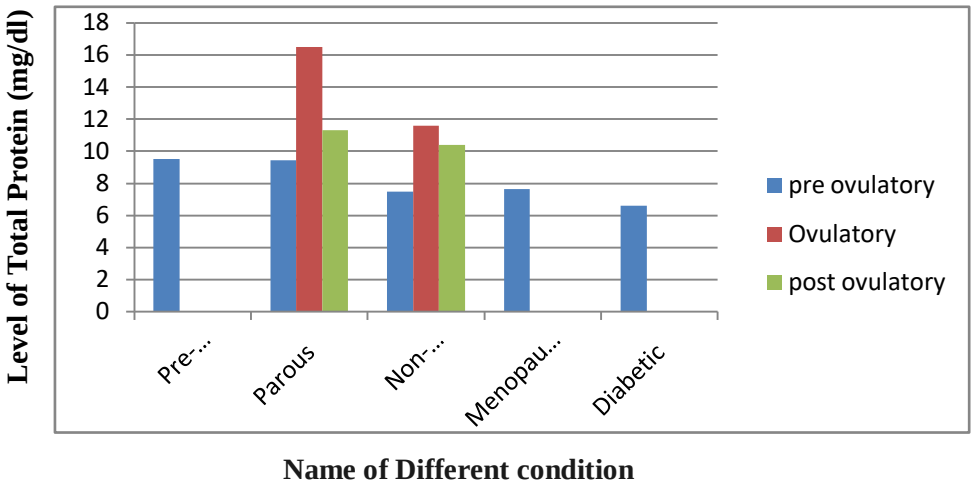
II. Materials and Methods

The studies were performed in 63 different human female volunteers of age group (7 to 45yrs.) categorizedas prepubertal, parous, non-parous, menopausal and diabetogenic. The human female unstimulated whole saliva was collected during various periods of parousand non-parous volunteers, viz. pre-ovulatory, ovulatory and post ovulatory phases and also from pre-pubertal, menopausal and diabetogenic stages and were stored at -70°C for further use (Navazesh and Christensen, 1982). Subjects were asked not to swallow any saliva during the collection period. Saliva volume was measured with the tube sealed and then frozen in dry ice until taken back to the laboratory for processing. All the saliva samples of different category of female subjects were placed into salivate tubeusing a natural cotton swab insert and centrifuged at 400gfor 10 minutes at 4°C. The Total protein was measured as per method of Lowry *et al.,* 1951 by Folin-ciocalteau phenol reagent.

III. Results & Discussion

Table 1: Level of Total Protein in saliva of different conditions in human female subjects

Sl No.	Name of different conditions with symbols	Level of Total Protein(mg/dl), Mean and SE of 6 samples	P-Value
1	Pre- pubertal –(a)	9.51 ± 0.153	
2	Parous Pre-ovulatory –(b) Ovulatory – (c) Post ovulatory –(d)	 9.40 ± 0.184 16.50 ± 0.149 11.28 ± 0.181	 a to c – (p<0.001) HS a to e – (p<0.001) HS a to f – (p<0.001) HS a to g – (p<0.01) HS
3	Non- Parous Pre-ovulatory –(e) Ovulatory – (f) Post ovulatory –(g)	 7.47 ± 0.264 11.58 ± 0.147 10.38 ± 0.153	 b to e – (p<0.001) HS c to f – (p<0.001) HS d to g – (p<0.01) S
4	Menopausal –(h)	7.61 ± 0.150	a to h – (p<0.001) HS b to h – (p<0.001) HS c to h – (p<0.001) HS d to h – (p<0.001) HS f to h – (p<0.001) HS g to h – (p<0.001) HS
5	Diabetogenic – (i)	6.58± 0.164	a to i – (p<0.001) HS b to i – (p<0.001) HS c to i – (p<0.001) HS d to i – (p<0.001) HS e to i – (p<0.02) S f to i – (p<0.001) HS g to i – (p<0.001) HS h to i – (p<0.01) HS



Figure

As per our findings a highly significant increased salivary total protein in parous and non-parous ovulatory and post ovulatory phase of menstrual cycle in comparison to prepubertal salivary total protein might be due to the diminished level of ovarian steroid estrogen & progesterone hormone in pubertal stage of female subject and active secretion and synthesis of steroid hormone in parous & non- parous human female subjects. A highly significant decreased level of salivary protein in non-parous preovulatory, ovulatory, post ovulatory phases of menstrual cycle than parous women phases might be an indication of disturbed hormone level in non- parous women. Earlier report of Alagendran *et al.,* 2013 indicated that protein range was highest at ovulatory phase than pre and post ovulatory phases due to estrogen peak. Thus, increased salivary protein level may be estrogen dependent. He further observed that total protein level in saliva were maximum during ovulation and minimum during post ovulatory phase. But in menopausal women's saliva the total protein showed as decreased level due to physiological endocrine-logical changes in menopausal women. As earlier report of Ambatipudi *et al.,* 2009 indicated that age related changes in protein abundance were observed. But in diabetogenic women a significant & highly significant increased level of salivary protein in comparison to prepubertal, parous and non-parous reproductive cycle phases salivary protein and from menopausal women might be due to disturbed metabolic activity in diabetic condition of women might be due to reduced salivary fluid secretion and various range of underlying pathogenic factors. Earlier report of Antonio *et al.,* 2004 support our findings that diabetic patients were attributed to reduced salivary fluid secretion. Our findings are consistent with the findings of Dodds and Dodds 1997 that increased salivary protein were observed in diabetic patients. Twetman *et al.,* 2002 also observed increased salivary total protein level in type -1 diabetic human female subjects than type-2.

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