INCREASED LEVELS OF SALIVARY INSULIN-LIKE GROWTH FACTOR-I (IGF-I) AND INSULIN LIKE GROWTH FACTOR BINDING PROTEINS-I AND 3 (IGFBP-I AND IGFBP-3) IN PATIENTS WITH PERIODONTITIS

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ABSTRACT

IGF-I (insulin-like growth factor-I) is probably the most potent anticatabolic and anabolic hormone. There are recent data in vivo and in vitro in animals according to which apoptosis of cells can be stopped by IGF-I. Insulin like growth factor binding protein-I (IGFBP-I) and insulin-like growth factor binding protein-3 (IGFBP-3) are the best characterized of circulating insulin-like growth factor binding proteins. IGFBPs are not only present in serum but are also produced locally by many different cells where they may have important functions in binding and/or targeting IGF-I for the respective receptors. The aim of this study was to measure serum IGF-I and its binding proteins versus

their levels in whole saliva in 30 patients with periodontitis and to compare these with the levels found in 30 healthy age- and sex matched controls. Serum insulin and IGFBP-3 were measured using immunoradiometric assay (IRMA) method while serum growth hormone, IGF-I, and IGFBP-I have been estimated utilizing ELISA method. Salivary IGF-I and IGFBP-1 have been measured using ELISA procedure while salivary IGFBP-3 has been measured by IRMA method. The serum levels of insulin, human growth hormone, IGF-I, IGFBP-I and -3 have not shown significant differences between the patients and healthy subjects. Salivary total protein was significantly higher in patients than in healthy controls.

The IGF-I, IGFBP-I and IGFBP-3 were significantly increased by almost one and half-fold concentrations in the whole saliva of periodontitis patients in comparison to controls ($P < 0.01$, $P < 0.05$, and $P < 0.05$ respectively). No significant correlation was found between salivary IGFBP-I and IGFBP-3 and their corresponding measured parameters in serum while a positive correlation ($P < 0.01$) between serum and salivary levels was observed only for IGF-I. On the other hand, significant positive correlations were found between salivary IGF-I and IGFBP-3 ($r = 0.36$; $P < 0.05$). These results indicate that synthesis and secretion of some proteins including IGFBP-I and IGFBP-3 are enhanced in glandular saliva from periodontitis subjects.

In conclusion; this study has demonstrated increased levels of IGF-I and its binding proteins IGFBP-I and IGFBP-3 in whole saliva of periodontitis patients. Also, there is a significant positive correlation between serum and salivary IGF-I levels.

INTRODUCTION

Periodontitis is a destructive inflammatory disease of the supporting tissues of the teeth. This disease is

primarily related to chronic plaque accumulation. Putative periodontopathic bacteria are suspected to play a role in the periodontal disease process. These bacteria release proteolytic enzymes that degrade salivary proteins, immunoglobulins, and collagen type I (Mayrand and Hok, 1988). Furthermore, these pathogenic bacteria provoke an immune response that results in the release of cytokines which trigger polymorphonuclear leucocytes, macrophages, fibroblasts, keratinocytes and osteoclasts. These host cells release proteinases which may degrade extracellular matrix components e.g. the highly resistant collagen fibres (Hanssen, 1993).

Cytokines play crucial roles in the maintenance of tissue homeostasis, a process which requires a delicate balance between anabolic and catabolic activities. In particular, growth factors such as fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), insulin-like growth factor (IGF), transforming growth factor-beta (TGF-beta) are thought to play important roles in modulating the proliferation and/or migration of structural cells in the periodontium and the production of various extracellular matrices by these cells (Okada and Muraka-

mi, 1998).

Stiles and coworkers (1979) have put forward the hypothesis that growth factors complement each other in their effects on the mitotic cycle of cells. Thus PDGF, EGF, FGF and other factors may act to render cells competent for the action of IGFs, which were termed progression factors. Nixon and Green (1984), have put forward a hypothesis according to which preadipocytes at an early state of differentiation would be rendered competent by growth hormone (GH) to respond to IGF-I to replicate.

In all these instances, growth factors or GH would deliver an important message to a cell in a stage of early differentiation and render it responsive to the mitogenic effect of IGF (Froesch et al., 1997). Insulin-like growth factors (IGFs) belong to a family of polypeptide hormones, also called somatomedins (Humbel, 1990). Most IGFs circulate in blood bound to a family of binding proteins (IGFBPs); six of which have been cloned and sequenced. These IGFBPs are released by different types of cells and may either augment or inhibit the action of IGFs. IGFBP-I and IGFBP-3 are the best characterized

of the circulating IGFBPs (Jones and Clemmons, 1995).

Quantitatively, IGFBP-3 is the most abundant in serum (Zapf et al., 1989). Approximately 85% of total serum IGF-I and II circulate in the form of a heterotrimer with a molecular mass of 150 Kd consisting of one molecule each of IGF, IGFBP-3 and acid-labile subunit (ALS) (Baxter and Martin, 1989). IGF-I in serum is mostly produced by the liver, as is ALS. An excess of the latter is present in the serum as the free molecule. IGFBP-3 in serum stems probably mostly from endothelial cells and is present under physiologic conditions in the 150-Kd complex together with IGF and ALS. When GH is lacking, less ALS is produced, and IGF-I may then circulate as a 50-Kd complex together with BP-3 and BP-2. Whereas the 150-Kd complex is not readily available to tissues and cells because the endothelium constitutes a barrier, free IGF-I as well as that present in the 50-Kd complex, together with BP-3 and other BPs, readily diffuse out the blood into the interstitial fluid and can then become active (Froesch et al., 1997). IGFBP-3 may also target IGFs to specific cells. On the other hand, IGFBPs are not only present in

serum but are also produced locally by many different cells where they may have important functions in binding and/or targeting IGF-I to the respective receptors (Schmid et al., 1992 and Peter et al., 1993).

IGF-I is released into the blood mostly by the liver (Schwander et al., 1983), and reaches target cells in the classic endocrine manner. However, it is also produced by peripheral cells, which are classic effector cells of IGFs: chondrocytes, osteoblasts, endocrine and many other cells (Adoshi et al., 1985; Nilsson et al., 1986; Ernst and Froesch, 1988 and Hammermann, 1989). The secreted IGF-I leads to increased collagen synthesis and cell differentiation (Froesch et al., 1997).

Halimi et al. (1994) found that the IGF-I levels in serum and saliva are somatotropin-dependent in acromegaly. However, Chaurasia et al. (1994) demonstrated that unlike IGF-I in serum, IGF-I secreted into the gastrointestinal lumen is not bound to binding proteins. Since the growth factor is not protein bound, its concentration in the gut lumen may be high enough to exert biological activity. Also, salivary

IGF-I concentrations were 100- to 200-fold less than plasma IGF-I levels (Ryan et al., 1992). IGF-I can stimulate the DNA synthesis of periodontal ligament fibroblasts, likely via binding to high affinity cell surface receptors. The effect of IGF-I was not enhanced by human growth hormone (Blom et al., 1992). Recombinant human insulin-like growth factor introduced into debrided lesions of experimentally induced periodontitis in monkeys induced predictable, clinically significant gains in new periodontal attachment (Rutherford et al., 1992).

Insulin-like growth factor-I and their binding proteins (BPs) IGFBP-I and IGFBP-3 have been assayed and characterized in many biological fluids as pleural fluid (Le Bouce et al., 1997). Also, Gargosky et al. (1992) have characterized and quantified IGFBP-3 in fetal cord serum, follicular, peritoneal, amniotic fluid, seminal plasma, and cerebrospinal fluid (CSF).

The aim of this study is to measure insulin-like growth factor-I (IGF-I) in whole saliva and in serum of patients with periodontitis and in matched healthy subjects and to find any correlation between salivary IGF-I and serum IGF-I and its binding pro-

teins (IGFBP-1 and IGFBP-3).

analysed for IGF-1, IGFBP-1, IGFBP-3 and total protein.

SUBJECTS AND METHODS

A group of 30 periodontally healthy subjects and another group of 30 periodontitis subjects were clinically characterized by measurement of various clinical parameters. The healthy group consisted of 17 men and 13 women, aged between 23 and 47 years (mean 37 ± 10). The periodontitis group consisted of 30 subjects 16 men and 14 women aged between 24 and 48 years (mean 38 ± 9). The subjects of this study were chosen from subjects attending the Oral Medicine Outpatient Clinic at the Dental Hospital of Mansoura University.

Collection of whole saliva

All mixed saliva samples were taken prior to the clinical examination at random points of time between 10.00-11.00 am 2 to 3 h after breakfast and after oral hygiene (Güven et al., 1996). Human whole saliva was collected by spitting, without stimulation into an ice-cooled vessel. To remove cells and bacteria to prevent degradation of salivary proteins, whole saliva was immediately cleared by centrifugal force (6000 rpm for 5 minutes) at room temperature and the supernatant was stored frozen at -20°C until

Blood collection

Fasting Blood samples (10ml) were drawn into polypropylene tubes. Serum was separated after centrifugation at 6000 rpm for 10 minutes, divided into aliquots and stored immediately at -20°C until used for measurement of IGF-1, IGFBP-1, IGFBP-3, insulin and growth hormone.

Routine liver function tests including serum albumin were done to exclude nutritional deficiency or liver disease which may affect IGF-I. Also serum creatinine was done to exclude renal disease.

Salivary protein determination

Salivary protein was measured using the method of Lowry et al. (1951). The principle of the method is that a copper-tartrate complex is allowed to react with the protein in alkaline solution. The protein-copper complex can reduce phosphomolybdate to form a blue substance.

Quantitative determination of IGF-I

The insulin-like growth factor-1 (IGF-1) was determined by an Enzyme-linked Immunosorbent Assay

(ELISA) Kit Supplied from Diagnostic Systems Laboratories, Inc., Webster, Texas, U.S.A. It is an enzymatically amplified "one step" sandwich-type immunoassay. The assay includes a simple extraction step in which IGF-1 is separated from its binding protein. This step is considered to be essential for accurate determination of IGF-1 (Daughaday and Rotwein, 1989 and Lee et al., 1990).

Determination of IGFBP-I

Total insulin-like growth factor binding protein-I (IGFBP-I) ELISA kit is an enzymatically amplified "two-step" sandwich-type immunoassay (Khosravi et al., 1997) supplied from Diagnostic Systems Laboratories Inc., Webster, Texas, USA.

Determination of IGFBP-3

The procedure employs a two site immunoradiometric assay (IRMA) principle described by Miles et al. (1974). The IRMA is a non-competitive assay in which the analyte to be measured is "sandwiched" between two antibodies. The first antibody is immunized to the inside walls of the tubes. The detected sensitivity limit of the assay was 0.5 ng/ml. The other antibody is radiolabelled for detection. (Gargosky et al.

1992; Le Bouce et al., 1997 and Corkins et al., 1998) (Diagnostic Systems Laboratories Inc. Webster, Texas, USA).

Quantification of insulin

The procedure employs a two site immunoradiometric assay (IRMA) principle (Brismar et al., 1994) (Diagnostic Systems Laboratories, Inc. Webster, Texas, USA).

Determination of human growth hormone

The method used is a "one-step" immunoenzymatic assay based on formation of a "sandwich" between the analyte to be detected and two specific monoclonal antibodies directed to different epitopes on the HGH molecule. The capture antibody is conjugated to biotin, while the second antibody, used to reveal the reaction, is labelled with horseradish peroxidase (HRP) (Hunter, 1976) (Sorin Biomedica Diagnostics, Vercelli, Italy).

Statistical analysis

Baseline clinical and biochemical results of this study were analyzed by student's T-test. Pearson's correlation coefficient was calculated for clinical and biochemical data, values of $P \leq 0.05$ were accepted as Statistically

significant. The statistical Analysis Systems (SAS Institute, Cary, NC) was used for all analyses.

RESULTS

Table (1) shows that there is no significant difference between healthy subjects and periodontitis patients as regards the serum levels of albumin, SGPT, creatinine, insulin, growth hormone, IGF-I, IGFBP-I and IGFBP-3.

The level of total protein in the whole saliva was significantly higher in periodontitis patients than in healthy subjects (table 2).

Table (2) also shows that IGF-I, IGFBP-I and IGFBP-3 levels in whole saliva were significantly higher in periodontitis subjects compared to healthy controls ($P < 0.01$, < 0.05 , < 0.05 respectively).

Correlations

No correlation between serum

IGF-I and the measured biochemical parameters in serum could be detected among all studied individuals. While serum IGFBP-I shows significant negative correlation with serum IGFBP-3 and albumin, it shows significant positive correlation with serum insulin. On the other hand, serum albumin is positively correlated with IGFBP-3 and negatively correlated with HGH (Table 3).

Table (4) Shows that salivary IGFBP-I has no significant correlation with any measured salivary biochemical parameter. Salivary IGFBP-3 shows significant positive correlation with both IGF-1 and total protein (Table 4). Interestingly, no significant correlation between salivary IGF-I and its binding proteins versus their serum levels, while there is a significant positive correlation between salivary IGF-I and serum IGF-I ($r = 0.69$; $P < 0.01$) (Table 5).

Table (1) : Comparison of serum biochemical parameters in periodontitis patients versus control group .

Parameters	Healthy (n = 30)		Periodontitis (n = 30)		Significance
	mean $\pm$ SD	range	mean $\pm$ SD	range	
Albumin gm/dL	4.6 $\pm$ 0.5	3.9-5.1	4.65 $\pm$ 0.6	3.9-5.2	NS
SGPT IU/ml	32 $\pm$ 6.1	25-37	33 $\pm$ 5.7	26-37	NS
Creatinine mg/dL	0.82 $\pm$ 0.3	0.6-1.0	0.87 $\pm$ 0.2	0.6-1.1	NS
S. insulin uIU/ml	4.35 $\pm$ 0.6	2.3-6.0	4.52 $\pm$ 0.7	2.4-6.3	NS
Growth hormone ng/ml	1.35 $\pm$ 0.5	0.6-1.5	1.4 $\pm$ 0.44	0.6-1.6	NS
IGF-1 ng/ml	158 $\pm$ 12.3	136-181	162 $\pm$ 13.9	130-195	NS
IGFBP-1 ng/ml	6.4 $\pm$ 2.1	2.9-9.6	6.7 $\pm$ 2.6	2.7-9.8	NS
IGFBP-3 ng/ml	2970 $\pm$ 778	1989-4200	3001 $\pm$ 695	1970-4305	NS

NS : non significant (p>0.05)

Table (2) : Comparison of biochemical parameters in whole saliva of healthy and periodontitis subjects .

Parameters	Healthy (n = 30)		Periodontitis (n = 30)		P
	mean $\pm$ SD	range	mean $\pm$ SD	range	
Total protein mg/ml	0.94 $\pm$ 0.39	0.49-1.75	1.42 $\pm$ 0.56	0.52-2.51	<0.05
IGF-1 ng/ml	4.01 $\pm$ 0.5	3.9-6.2	6.7 $\pm$ 0.4	4.1-7.9	<0.01
IGFBP-1 ng/ml	2.1 $\pm$ 0.37	1.5-2.8	3.0 $\pm$ 0.4	2.2-3.7	<0.05
IGFBP-3 ng/ml	926 $\pm$ 120	720-1205	1549 $\pm$ 198	1210-1820	<0.05

p<0.05 : Significant

P<0.01 : Highly significant .

Table (3) : Correlation between different hormonal and biochemical parameters in the serum of studied subjects .

Parameters	S.IGF-1	S.IGFBP-1	S.IGFBP-3	S.Insulin	S.HGH
S. IGFBP-1	$r = 0.080$				
S. IGFBP-3	$r = -0.06$	$r = -0.484^*$			
S. insulin	$r = 0.10$	$r = 0.396^*$	$r = 0.185$		
S. HGH	$r = -0.12$	$r = 0.127$	$r = -0.240$	$r = -0.125$	
S.Albumin	$r = 0.180$	$r = -0.781^{**}$	$r = 0.81^{**}$	$r = -0.321$	$r = -0.465^*$

* $p < 0.05$ ** $P < 0.01$ **Table (4) :** Correlation between different measured salivary parameters in the studied subjects .

Parameters	IGF-1	IGFBP-1	IGFBP-3
IGFBP-1	$r = 0.10$		
IGFBP-3	$r = 0.36^*$	$r = -0.10$	
Total protein	$r = 0.26$	$r = 0.09$	$r = 0.79^{**}$

* : $p < 0.05$ ** : $P < 0.01$ **Table (5) :** Correlation between serum IGF-1 and its binding proteins versus their levels in whole saliva in the studied subjects .

Parameters	Salivary IGF-1	Salivary IGFBP-1	Salivary IGFBP-3
Serum IGF-1	$r = 0.69^{**}$	$r = 0.07$	$r = 0.11$
Serum IGFBP-1	$r = 0.10$	$r = 0.09$	$r = 0.08$
Serum IGFBP-3	$r = -0.12$	$r = -0.21$	$r = 0.21$

** : $P < 0.01$

DISCUSSION

IGF-1 and the other principal insulin-like growth factor IGF-II are structurally related to proinsulin, however, unlike insulin, they circulate bound to a family of binding proteins (IGFBPs) (Sara and Hall, 1990). These binding proteins have profound effects on the half-life, distribution, bio-availability, and bioactivity of IGFs (Baxter and Martin, 1989 and Herrington, 1991).

The inverse relationship between insulin and IGFBP-1 is well described (Holly et al., 1988) and was observed after administration of either placebo or IGF-1 (Cheetham et al., 1994). IGFBP-1 transcription and production in the liver is believed to be primarily regulated by portal insulin, with an increase in circulating insulin levels resulting in a reduction in the concentration of IGFBP-1 within 2 hours (Suikkari et al., 1989). IGFBP-1 may then act as a regulator of IGF-1 action, inhibiting IGF bioactivity by increased circulatory binding when insulin levels are low and possibly transporting the peptide to the tissues after meals when insulin levels are high (Holly, 1991).

Regulation of plasma or tissue levels of insulin-like growth factors

(IGFs) depends on a number of factors. Among these, nutrition and growth hormone (GH) have been shown to be of prime importance in several species (Clemmons and Van Wyk, 1981, Clemmons et al., 1981, Pell and Gilmour, 1993).

The main purpose of this study was to search for differences in IGF-I and its binding proteins in output of salivary secretion between periodontally healthy and diseased subjects. Previous studies (Henskens et al., 1993a, Henskens et al., 1993b and Henskens et al., 1994) have reported that the total protein and albumin concentrations were higher in whole saliva from periodontitis subjects. The present study deals with the biochemical analysis of whole saliva and correlates their levels with the measured parameters in serum particularly IGF-I, IGFBP-I and IGFBP-3.

In general, the major factors affecting the protein concentration and composition of whole saliva are the salivary flow rate, the protein concentration of the contributing glandular salivas and the contribution of crevicular fluid proteins (Henskens et al., 1996). The increased protein concentration in periodontitis subjects cannot

be attributed to a changed flow rate, since in many previous studies no statistically significant differences in mean saliva flow rate was found (Aguivre et al., 1992, Shern et al., 1993 and Henskens et al., 1996). Moreover, no correlation was found between flow rate and any of the biochemical or clinical parameters (Henskens et al., 1996). The mean salivary level of total protein of the present periodontitis group was significantly higher than in the control group ($P < 0.05$) (Table 2). These results are in agreement with previous studies (Meechan, 1983 and Basu et al., 1984). The elevated salivary total protein levels are most likely due to enhanced synthesis and secretion by individual glandular salivas (Basu et al., 1984, Henskens et al., 1996). Similarly, of the glandular proteins tested; IGF-I, IGFBP-1 and IGFBP-3 were present in almost one and half-fold higher concentrations in whole saliva of periodontitis patients (Table 2). Although the exact mechanism of this increase and the release in saliva is unclear, it may be attributed to enhanced synthesis of some acinar proteins by salivary glands of periodontitis patients and thereby increasing the protective potential of saliva. The results of this study rather support

earlier presumptions, that IGF-I may play important roles in modulating the proliferation of structural cells in the periodontium (Okada and Murakami, 1998). The present study rather support earlier suggestions that IGF-I is produced by peripheral cells (Adoshi et al., 1985 and Hammermann, 1989) and IGFBP-3 in serum stems probably mostly from endothelial cells (Froesch et al., 1997).

No correlations were found between salivary measured biochemical parameters and the corresponding measured parameters in serum except for IGF-I (Table 5). These results confirm that periodontitis is a local manifestation. On the other hand, significant positive correlation was found between salivary IGF-I and its binding protein (IGFBP-3) ($r = 0.36$, $P < 0.05$) (Table 4). Hormia et al. (1993) reported higher concentration of epidermal growth factor in glandular salivas from juvenile periodontitis subjects. The present and previous studies (Henskens et al., 1993a and Henskens et al., 1994) also indicate that levels of some proteins are enhanced in glandular salivas from periodontitis subjects. The effect of external stimuli on protein synthesis in the human salivary glands has not been es-

established experimentally. However, many animal studies have provided evidence that expression of certain salivary proteins in rodents is influenced by various external stimuli (Yagil and Baska, 1986). They showed that irritating actions (incisor amputation) produced dramatically more of glandular production of cystatin S in rats. On the basis of the results of our study, it can be hypothesized that similar regulatory mechanisms might act in human beings as well.

Conclusion

The present study, to our knowledge, is the first one to comprehensively measure the IGF-I, IGFBP-I and IGFBP-3 in whole saliva of periodontitis patients. In conclusion, this study has demonstrated increased levels of IGF-I and its binding proteins in whole saliva of periodontitis patients. Also, there is a significant positive correlation between serum and salivary IGF-I levels. Although the exact mechanism of this increase and the release in saliva is unclear, it may be attributed to enhanced synthesis of some acinar proteins by salivary glands of periodontitis patients and thereby increasing the protective potential of saliva. Whether the concentrations of other growth factors or oth-

er glandular derived proteins are also changed in saliva of periodontitis patients remains to be elucidated.

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زيادة مستوى عامل النمو شبيه الأنسولين-١ والبروتين المرتبط بعامل النمو شبيه الأنسولين-١، ٣ فى لعاب مرضى التهاب الغشاء الحول سنى

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أن عامل النمو شبيه الأنسولين-١ يعتبر من أقوى الهرمونات المساعدة لعملية الأيض البنائى ومضاد لعملية الأيض الهدمى. كما يعتبر كل من البروتين المرتبط بعامل النمو شبيه الأنسولين-١، ٣ الأكثر تميزاً فى الارتباط بعامل النمو شبيه الأنسولين-١ فى الدورة الدموية. كما تنتج هذه البروتينات أيضاً بواسطة خلايا أخرى مختلفة لتلعب دوراً هاماً فى الارتباط بعامل النمو شبيه الأنسولين-١ أو توجيهه إلى مستقبلات خاصة.

أستهدفت هذه الدراسة قياس مستوى عامل النمو شبيه الأنسولين-١ والبروتينات المرتبطة به فى كل من المصل واللعاب لأشخاص أصحاء ومرضى مصابين بالتهاب الغشاء الحول سنى ومحاولة إيجاد علاقة بين مستوى كل من عامل النمو شبيه الأنسولين-١ والبروتينات المرتبطة به فى مصل ولعاب هؤلاء الأشخاص .

إشتملت هذه الدراسة على ٦٠ شخصاً تم تقسيمهم إلى مجموعتين الأولى مكونة من ٣٠ شخصاً يعانون من التهاب الغشاء الحول سنى والمجموعة الأخرى مكونة من ٣٠ شخصاً من الأصحاء فى نفس المرحلة السنبة ولا يعانون هذا المرض أو أى أمراض أخرى ظاهرة .

وقد تم إختيار الأشخاص المشاركون فى هذا البحث من المترددى على العيادة الخارجية لطب الفم والأسنان بكلية طب الأسنان - جامعة المنصورة .

وقد تم تجميع عينات اللعاب وأخذ عينات الدم من كل شخص مشارك في هذا البحث وفصل المصل وحفظ العينات عند درجة -٢٠ درجة مئوية حتى تم قياس الانتى :-

فى المصل : تم قياس مستوى هرمون الأنسولين والبروتين المرتبط بعامل النمو شبيه الأنسولين -٣ بواسطة طريقة المناعة الاشعاعية بينما تم قياس مستوى هرمون النمو وعامل النمو شبيه الأنسولين-١ والبروتين المرتبط بعامل النمو شبيه الأنسولين-١ بطريقة الاليزا.

فى اللعاب : تم قياس مستوى عامل النمو شبيه الأنسولين -١ والبروتين المرتبط بعامل النمو شبيه الأنسولين -١ بطريقة الاليزا بينما تم قياس البروتين المرتبط بعامل النمو شبيه الأنسولين-٣ بطريقة المناعة الاشعاعية .

وقد أوضحت نتائج هذه الدراسة زيادة مستوى كل من عامل النمو شبيه الأنسولين-١ والبروتينات المرتبطة بعامل النمو شبيه الأنسولين-١، ٣ فى لعاب المرضى المصابين بالتهاب الغشاء الحول سنى مقارنة بالأصحاء. كما تبين وجود علاقة إيجابية ذات دلالة إحصائية بين مستوى عامل النمو شبيه الأنسولين-١ فى المصل ونظيره فى اللعاب .

كذلك تبين وجود علاقة إيجابية ذات دلالة إحصائية بين مستوى عامل النمو شبيه الأنسولين-١ والبروتين المرتبط بعامل النمو شبيه الأنسولين-٣ فى اللعاب .

وقد أمكن الاستدلال من هذه النتائج زيادة تصنيع وإفراز بعض البروتينات ومن بينها البروتينات المرتبطة بعامل النمو شبيه الأنسولين-١، ٣ فى لعاب مرضى التهاب الغشاء الحول سنى .