

EXPRESSION OF IGF-1 (INSULIN-LIKE GROWTH FACTOR-1) RECEPTOR ON  
GINGIVAL TISSUE SAMPLES IN DIABETIC PATIENTS AND CONTROLS

By

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A THESIS PRESENTED TO THE GRADUATE SCHOOL  
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT  
OF THE REQUIREMENTS FOR THE DEGREE OF  
MASTER OF SCIENCE

UNIVERSITY OF FLORIDA

2004

## ACKNOWLEDGMENTS

I would like to express my gratitude to those that have helped in completion of this research project. I would like to thank my committee, Dr. Joseph Katz, Dr. Herbert Towle, and Dr. Frederic Brown. I would also like to thank my program director, Dr. Gregory Horning. Special thanks go to Dr. Donald Cohen, Dr. Juliana Robledo, Dr. Indraneel Bhattacharyya and the entire Department of Oral Medicine and Diagnostic Sciences for making their resources and knowledge available to me. I would also like to thank my wife, Dr. Kimberly Jones-Rudolph for her unwavering love and support.

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Abstract of Dissertation Presented to the Graduate School  
of the University of Florida in Partial Fulfillment of the  
Requirements for the Degree of Master of Science

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May 2004

Chair: Herbert J. Towle  
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The purpose of this investigation is to evaluate the expression of Insulin-Like Growth Factor-1 Receptor (IGF-1) in gingival tissue samples of self reported diabetic patients versus controls. The thesis proposed is that there is an up-regulation of the IGF-1 receptor in the gingival tissues of diabetics versus controls. Previous investigations have shown the up-regulation of IGF-1 receptor is associated in the pathogenesis of diabetes. Until this study, nobody has examined gingival tissues for these receptors and up-regulation.

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, blood vessels, and the oral cavity. Patients suffer delayed wound healing and increased risk for infections. People with diabetes are also more likely to have periodontal disease than non-diabetics because

of heightened susceptibility to infections. Those patients who do not have their diabetes under control are especially at risk. Periodontal diseases are serious bacterial infections that destroy the attachment fibers and supporting bone that maintain teeth in the oral cavity. Left untreated, these diseases can lead to tooth loss.

The methods and materials utilized in this study involved collecting gingival tissue samples from diabetic patients and controls. Those samples were then prepared for immunohistochemical preparation. After the samples were prepared they were then scored and graded based on the intensity of stain. A strong staining intensity correlated with a higher number of receptors and was interpreted as an up-regulation.

The present study has shown the expression of IGF-1 receptors on both gingival tissue samples of Type 2 diabetics with varying degrees of periodontal disease and gingival tissue samples of healthy non-diabetic subjects with periodontal disease. Although no statistical differences between the groups could be established, a trend for increased expression of IGF-1 receptors in diabetics and a decreased expression in controls was apparent. The preliminary findings of this study are significant in the confirmation of the presence of IGF-1 receptors in gingival tissue. Although presence of up-regulation can not be statistically confirmed, this study gives reason for further investigation in this area.

## CHAPTER 1 INTRODUCTION

### **Background**

Diabetes is a group of diseases characterized by high levels of blood glucose resulting from defects in insulin production, insulin action, or both. Insulin is a hormone that is needed to convert sugar, starches and other food into energy needed for daily life. The cause of diabetes continues to be unknown, although both genetics and environmental factors such as obesity and lack of exercise appear to play roles. There are currently 18.2 million people in the United States, or 6.3% of the population, who have diabetes [1]. While an estimated 13 million have been diagnosed, 5.2 million people (or nearly 33%) are unaware that they have the disease [1, 2]. Systemic complications of Diabetes Mellitus (DM) include retinopathy, nephropathy, neuropathy, increased susceptibility to infection, increased risk of periodontal disease, and altered wound healing.

This paper will focus on the relationship of DM and oral complications, in particular periodontal diseases. Previous studies have revealed an up-regulation of insulin-like growth factor-1 receptors (IGF-1) involved in the pathogenesis of DM in various tissues, but until now no one has investigated gingival tissues. This study will examine gingival tissue samples from self-reported diabetic patients and self-reported non-diabetic controls to confirm the presence of the IGF-1 receptors and determine whether there is an up-regulation in those patients with diabetes.

### **Description of the Disease**

There are four clinical classifications of diabetes: Type I (resulting from B-cell destruction, usually leading to absolute insulin deficiency), Type II (resulting from a progressive insulin secretory defect on the background of insulin resistance), other specific types of diabetes (due to other causes, e.g. genetic defects in B-cell function, genetic defects in insulin action, diseases of the exocrine pancreas, drug or chemical induced) and Gestational Diabetes Mellitus (GDM) [3].

Both Type I and II DM are chronic diseases with Type I considered the most severe form of diabetes [4]. Type I DM occurs due to little or no production of insulin by the pancreas resulting in hyperglycemia and must be treated with insulin injections [5]. Type 1 DM was previously called insulin-dependent diabetes mellitus (IDDM) or juvenile-onset diabetes. Type 1 DM develops when the body's immune system destroys pancreatic beta cells, the only cells in the body that make the hormone insulin to regulate blood glucose. It is not clear whether a given environmental factor (e.g. a precise virus or a cow's milk component) plays an etiological role the development of type 1 DM [5]. Type 1 DM appears as a multifactorial disease. It is not known whether all factors intervene concomitantly in a given individual or separately in subsets of patients, explaining the clinical heterogeneity of the disease [5]. And, the mechanisms underlying the loss of tolerance to self beta-cell autoantigen(s) are still unknown [5]. This form of diabetes usually strikes children and young adults, although disease onset can occur at any age. Symptoms of Type I DM includes: increased thirst, increased urination, weight loss despite increased appetite, fatigue, nausea and vomiting. Type 1 DM may account for 5% to 10% of all diagnosed cases of diabetes [2]. Risk factors for Type 1 DM include autoimmune, genetic, and environmental factors [6, 7, 8, 9, and 10].

Type 2 DM was previously called non-insulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes. Type 2 DM may account for about 90% to 95% of all diagnosed cases of diabetes [2]. It usually begins as insulin resistance, a disorder in which the cells do not properly use insulin. As the need for insulin rises, the pancreas gradually loses its ability to produce insulin. Type 2 DM is associated with older age, obesity, family history of diabetes, prior history of gestational diabetes, impaired glucose tolerance, physical inactivity, and race/ethnicity. African Americans, Hispanic/Latino Americans, Native Americans, and some Asian Americans, Native Hawaiian, or other Pacific Islanders are at particularly high risk for Type 2 DM and is increasingly being diagnosed in children and adolescents [6, 10].

Table 1-1. Risk Factors Associated with Diabetes

| <b>Risk Factor</b> | <b>Description</b>   |
|--------------------|--|
| Older Age          | As people get older, they become less active and may gain excess weight. Over 65 years, the incidence of type 2 DM reaches 20%   |
| Obesity            | Body mass index (BMI) is an indication of whether your weight is in the healthy weight range in relation to your height. A BMI of 30 or greater is considered overweight   |
| Body Composition   | Weight is only part of the equation. Individuals who carry most of their weight in the trunk of their bodies, above the hips, tend to have a higher risk of diabetes than those of similar weight with a pear-shaped body, excess fat carried mainly in the hips and thighs. A waist measurement of more than 100 cm (39.5 inches) in men and 95 cm (37.5 inches) in women suggests an increased risk  |
| Family History     | Having a blood relative with type 2 DM increases the risk. If that person is a first-degree relative, the risk is even higher. Genes are responsible for many aspects of regulating blood glucose control, and problems with these genes or how they work under certain conditions, such as stress, inactivity or overweight, may be responsible for diabetes. The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) is planning the Diabetes Genome Anatomy Project, which will profile genes in all tissues relevant to diabetes, including fat, muscle, and kidney, to gain insight into the origin and development of diabetes and its complications. |

Table 1-1. Continued

| <b>Risk Factor</b>               | <b>Description</b>   |
|----------------------------------|--|
| Gestational Diabetes             | Some women develop gestational DM during pregnancy. It is more common when the baby is over 4kg (9lbs). Nearly 40 percent of the women who have diabetes during their pregnancy go on to develop type 2 DM later, usually within five to ten years of giving birth.  |
| Impaired Glucose Tolerance (IGT) | Occurs when the level of glucose in the blood is higher than normal but not in the diabetic range. An estimated one in ten progress to type 2 DM within five years.  |
| Polycystic Ovary Syndrome (PCO)  | PCO is a condition where a woman of childbearing age does not ovulate, or the eggs or ova are not released from the ovary. This causes cysts in the ovaries to develop and the level of male hormones, such as testosterone, to become elevated in the bloodstream. It is estimated that 30-50% of women with PCO will have impaired glucose tolerance or diabetes by the age of 30.   |
| Physical Inactivity              | Lack of aerobic exercise and weight training.  |
| Damage to the Pancreas           | Alcohol, trauma, pancreatitis, and perhaps some toxins are capable of damaging the pancreas.   |
| Race/Ethnicity                   | In some ethnic groups type 2 DM is more common and develops at an earlier age. Being of Aboriginal, African, Latin American, American Indian, Pacific Islander, or Asian ethnic ancestry increases the risk of developing of type 2 DM. This may be due to genetic differences, differences in eating habits and foods, and/or less physical activity. This is particularly the case when people migrate to live in a western culture and adopt the diet and lifestyle of the new country, or move from rural areas to the city. This often results in people consuming an increased intake of high fat convenience foods and leading a less active lifestyle. |

Source: About.com and the CDC: National Diabetes Fact Sheet 2002.

Gestational DM is a form of glucose intolerance that is diagnosed in some women during pregnancy. Gestational DM occurs more frequently among African Americans, Hispanic/Latino Americans, and Native Americans [6, 10]. It is also more common among obese women and women with a family history of diabetes. During pregnancy, gestational diabetes requires treatment to normalize maternal blood glucose levels to avoid complications in the infant. Gestational diabetes occurs in 4% of pregnant women [11] with no previous diabetes history and is usually self-correcting after pregnancy [12]. However, mothers with gestational diabetes are at a greater risk for developing Type II

DM in the future. In fact, approximately 40% of women that develop gestational DM during pregnancy develop Type II DM within 15 years of the pregnancy [12].

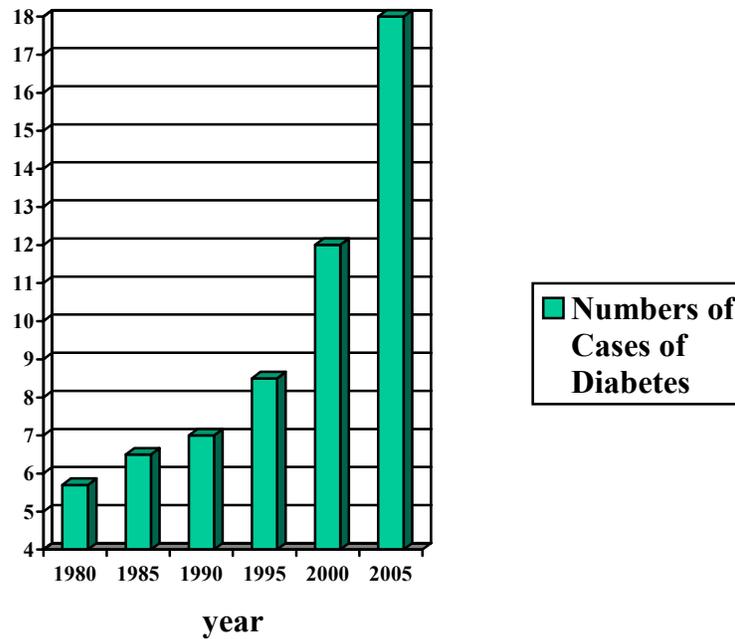


Figure 1-1: Number of Persons with All Forms of Diagnosed Diabetes, United States, 1980-2000, Centers for Disease Control and Prevention, National Center for Health Statistics, Division of Health Interview Statistics, data from the National Health Interview Survey [2].

As alarming as the rise in the numbers of diagnosed cases, there are many people who do not even know that they have the disease. There are currently 18.2 million people in the United States, or 6.3% of the population, who have diabetes [1, 2]. While an estimated 13 million have been diagnosed, 5.2 million people are unaware that they have the disease [1, 2].

### **Disease Diagnosis**

There are two different tests that are used in diabetes: screening tests and diagnostic tests. Screening tests are done on people who have no symptoms of the disease.

Diagnostic tests are done to confirm a diagnosis that is already suspected from the

patient's symptoms. Table 1-2 below describes criteria for screening individuals for diabetes and Table 1-3 outlines criteria used for the diagnosis of diabetes.

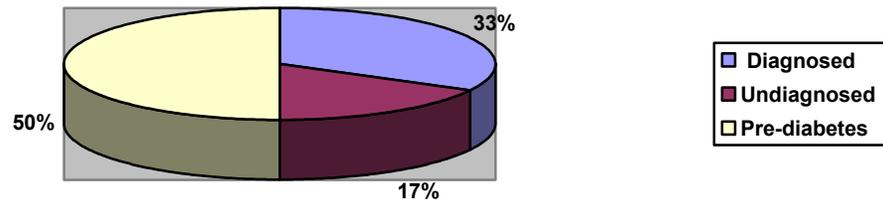


Figure 1-2: Percentages of Pre-diabetic, Diagnosed and Undiagnosed Diabetics in 2002 [13]

There are so many undiagnosed cases of diabetes, primarily due to the fact that diabetes is an insidious disease that one may have for decades without knowing it [13].

Diagnostic tests for diabetes include: oral glucose tolerance test (OGTT), fasting plasma glucose (FPG), and A1C. The OGTT is more sensitive than the other tests and more specific diagnostic test than FPG, but not very reproducible so it is used less frequently. Although FPG is less specific, it is less costly, easy to use and has high patient acceptance.

The OGTT performed having the patient fast for at least eight hours to test the patients fasting glucose level. After that baseline the patient receives 75 grams of glucose and blood samples are taken up to four times over a 2-3 hours time period to measure the blood glucose. In a person without diabetes, the glucose levels rise and then quickly fall. In diabetics the glucose levels rise higher than normal and do not fall as quickly.

Table 1-2. Criteria for Testing for Diabetes in Asymptomatic Adult Individuals [14]

1. Testing for diabetes should be considered in all individuals at age 45 years and above, particularly in those with a BMI  $\geq 25$  kg/m<sup>2\*</sup>, and, if normal, should be repeated at 3-year intervals.
2. Testing should be considered at a younger age or be carried out more frequently in individuals who are overweight (BMI  $\geq 25$  kg/m<sup>2\*</sup>) and have additional risk factors:
  - are habitually physically inactive
  - have a first-degree relative with diabetes
  - are members of a high-risk ethnic population (e.g., African-American, Latino, Native American, Asian-American, Pacific Islander)
  - have delivered a baby weighing >9 lb or have been diagnosed with GDM
  - are hypertensive ( $\geq 140/90$  mmHg)
  - have an HDL cholesterol level  $\leq 35$  mg/dl (0.90 mmol/l) and/or a triglyceride level  $\geq 250$  mg/dl (2.82 mmol/l)
  - have PCOS
  - on previous testing, had IGT or IFG
  - have other clinical conditions associated with insulin resistance (e.g. PCOS or acanthosis nigricans)
  - have a history of vascular disease

Table 1-3. Criteria for the Diagnosis of Diabetes [3]

1. Symptoms of diabetes and casual plasma glucose  $\geq 200$  mg/dl (11.1 mmol/l). Casual is defined as any time of day without regard to time since last meal. The classic symptoms of diabetes include polyuria, polydipsia, and unexplained weight loss.
- OR
2. Fasting Plasma Glucose (FPG)  $\geq 126$  mg/dl (7.0 mmol/l). Fasting is defined as no caloric intake for at least 8 h.
- OR
3. 2-h PG  $\geq 200$  mg/dl (11.1 mmol/l) during an Oral Glucose Tolerance Test (OGTT). The test should be performed as described by the World Health Organization (4), using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.

If the 2-hour glucose level is less than 140 mg/dl, and all values between 0 and 2 hours are less than 200 mg/dl indicates a normal result. Impaired glucose tolerance is when the fasting plasma glucose is less than 126 mg/dl and the 2- hour glucose level is between 140 and 199 mg/dl. Diabetic response is when two diagnostic tests done on different days show that the blood glucose level is high.

If the OGTT yields a positive result for diabetes a follow up test is the FPG, done after having fasted overnight (at least 8 hours). Normal fasting plasma glucose levels are less than 110 mg/dl. Fasting plasma glucose levels of more than 126 mg/dl on two or more tests on different days indicates a diagnosis of diabetes.

Another test that is used in diabetes is the glycated hemoglobin test; also know as glycohemoglobin (GHb), glycosylated hemoglobin, HbA1c, HbA1 or A1C. This test measures the amount of glucose available in the hemoglobin. Erythrocytes are permeable to glucose and have an average life span of 120 days. This lab tests reflects the previous 2-3 months of glycemic control. The A1C test has been shown to be a good predictor of the development of many of the chronic complications in diabetes and used to monitor patients who have already been diagnosed with diabetes. People with diabetes can have normal levels, so it is not a test that is used to diagnose patients. The A1C test is recommended to be done every three months until the patient is within the target range, then at least two times a year. The goal of therapy should be an A1C value of < 7% [12].

### **Oral Complications of Diabetes**

Both Type 1 and 2 DM are risk factors for periodontal diseases. Patients with Type 1 DM, especially those that have had the condition for a long duration, have been found to have more gingivitis and more deep periodontal pockets than controls [15, 16, and 17]. Uncontrolled or poorly controlled diabetes has been shown to be associated with

increased susceptibility to oral infections, including periodontitis [18, 19]. There have been several studies which have reported a significantly poorer periodontal health in Type 2 DM patients and some of these reports have provided epidemiologic parameter estimates of association and risk. The odds that have been reported for Type 2 diabetics to have greater risk of destructive periodontal disease are from 2.6 to 4.0 [20, 21, and 22]. There have also been two population-based surveys that have provided epidemiologic estimates of association for diabetes and attachment loss severity, with diabetic individuals being twice as likely to have more severe attachment loss as those without diabetes [23, 24].

Current evidence supports the fact that inferior glycemic control contributes to poorer periodontal health. Recent studies that have been published on the association between glycemic control and periodontal disease have shown that inadequate glycemic control is a significant factor associated with poorer periodontal health [25, 26, and 27]. The control of diabetes is directed at controlling the blood glucose levels within “normal limits”, and there is clear evidence that complications can be prevented by meticulous control of hyperglycemia [28, 29]. Monitoring the effectiveness of glycemic control is done by measuring the levels of glycosylated serum proteins, in particular glycosylated  $\alpha$ -hemoglobin (HbA1c), which because of its incorporation into the red blood cells gives an indication of the serum glucose levels over the preceding 2 to 3 months [30].

### **Study Rationale**

Insulin-like growth factors (IGFs) belong to a family of polypeptide hormones, also called somatomedins (“mediator of growth”) [31]. IGF-1 is a well-characterized basic peptide that has some unique characteristics and properties. It has growth-regulating,

insulin-like, and mitogenic activities [32]. It has a major, but not absolute, dependence on growth hormone (GH). IGF-1 has endocrine as well as paracrine functions. A paracrine mode of action occurs when a growth factor that is secreted by one cell has an effect on adjacent cells [32]. An endocrine mode of action is when a substance is produced in an endocrine gland, secreted into the blood stream, and acts at locations distant from its site of synthesis. IGF-1 is released into the blood by the liver and reaches target cells in the classic endocrine manner [32]. However, it is also produced by peripheral cells, which are classic effector cells of IGFs: chondrocytes, osteoblasts, endocrine, fibroblasts, as well as other cells [33, 34, 35, and 36]. Currently it is not known whether the endocrine or paracrine natures of IGFs are more important in the process of growth and differentiation of cartilage and bone, as well as other tissues [34].

IGF-1 was originally discovered based on its property of stimulating sulfation of proteoglycans that are present in cartilage [37]. It was later determined that it was an important stimulant of cartilage DNA synthesis [38]. This property was discovered while trying to develop in vitro assays for GH activity [39, 40]. When GH was added to cartilage in vitro, it was a poor stimulant of cartilage sulfation [39, 40]. But the administration of GH to hypophysectomized animals resulted in indication of a substance in serum that was a potent stimulant of cartilage sulfation [39, 40]. This suggested that a separate growth factor was induced in serum [39, 40]. Purification of this substance led to the determination of its primary amino acid sequence and to studies that showed that it could stimulate growth in whole animals [39, 40]. IGF-1 has significant amino acid sequence homology to pro-insulin. It is synthesized as a large precursor molecule and is proteolytically cleaved to release the biologically active monomer [41]. Models of the

three-dimensional structures of insulin, proinsulin, and IGF-1 visualize the similarity between the three molecules [42, 43]. The variability of the hydrophilic amino acid residues between insulin and IGF-1 is remarkable, a finding that explains why antibodies directed against insulin cross-react only very weakly with the IGF-I, and vice versa [44]. The similarity between the two molecules is much greater in the hydrophobic regions responsible for receptor binding, a finding that would explain why there is cross-reactivity between insulin and IGF-1 at the insulin and the IGF-1 receptor [44].

There are two major IGF receptors on cells; the type I, also called IGF-1 receptor, and the type II, also called IGF-II receptor [44]. The type I receptor is homologous to the insulin receptor [44]. It is a heterotetrameric glycoprotein which consists of two ligand-binding subunits called  $\alpha$ - and  $\beta$ - subunits [45]. Only the  $\beta$ -subunits have a transmembrane domain [45]. The  $\beta$ - subunit of the receptor is composed of a transmembrane domain that is followed by a long, intra-cytoplasmic domain [45]. This region contains intrinsic tyrosine kinase (TK) activity and critical sites of tyrosine and serine phosphorylation. [45]. The TK domain is 84% homologous to the insulin receptor TK domain [45]. The catalytic domain contains an adenosine triphosphate (ATP) binding motif and a catalytic lysine. Substitution for this lysine abolishes IGF-I stimulated biologic secretions [46]. Ligand binding to the  $\alpha$ - subunit triggers a conformational change and dimerization that leads to auto-activation [47, 48]. This, in turn, leads to transreceptor phosphorylation, wherein specific tyrosine's located on one  $\beta$ -subunit is transphosphorylated by the TK activity located on the paired  $\beta$ -subunit [49, 50]. This mode of TK activation that results in tyrosine auto-phosphorylation is similar to that which occurs in the insulin receptor [49, 50]. The IGF-1 receptor has the highest affinity

for IGF-1, followed by IGF-2, and the lowest for insulin [49, 50]. The affinity of insulin for binding to the IGF-1 receptor is 5 to 10% of that of IGF-1 [49, 50].

The IGF-1 receptor is omnipresent and has been shown to be present in all cell types derived from all three embryonic lineages [51, 52]. When animal tissues have been analyzed the IGF-1 receptor can be detected uniformly. To date human gingival tissue has not been studied. The hormonal regulation of IGF-1 receptor number has been analyzed in great detail [51]. Hormones such as GH, FSH (follicle-stimulating hormone), LH (luteinizing hormone), progesterone, estradiol, and thyroxin (T4), have been shown to increase receptor expression [51, 52]. Similarly, platelet-derived growth factor (PDGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), and angiotensin II up regulate expression of the IGF-1 receptor in specific cell types [53, 54]. Following hormone binding, there is a classic down regulation of receptor number with internalization of receptors [54]. However, possibly owing to Insulin-like growth factor binding proteins (IGFBPs), the rate of internalization of IGF-1 receptors is substantially less than that of other growth factors, such as EGF or insulin [54].

IGF-1 is present in all biological fluids almost entirely (95-99%) bound to a family of structurally related binding proteins (IGFBPs) [54]. Six IGFBPs have so far been identified and cloned [55]. Quantitatively, IGFBP-3 is the most abundant in serum [56]. Approximately 85% of total serum IGF-1 circulates 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) [57]. In this large complex IGF-1 has a half-life in humans of about sixteen hours, whereas with other IGFBPs the half-life is about twenty minutes [58]. The half-life of free IGF-1 is a few minutes, similar to that of insulin. Besides their IGF half-

life prolonging function, IGFbps may inhibit or enhance the activity of IGF-I [59, 60, 61]. They may also target IGFs to specific cells. 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-1 to the respective receptors [62, 63]. The IGFbps differ in their regulation and their affinities for IGF-1 [62, 63]. In addition to carrier proteins, there is increasing evidence that they act as potentiators or modulators of several complex physiological activities of IGF-1 [64].

The biologic activities of IGF-1 can be divided into two types of responses: rapid metabolic effects (insulin-like), and slower growth-promoting effects (mitogenic) [64, 65]. It has been demonstrated that these peptides act as mitogens for many cell types [66, 67]. In this capacity, IGF-1 appears to allow the cell to progress from G1 to S phase of the cell cycle. In fibroblasts, other factors such as PDGF or EGF are required to make the cell competent to traverse the cell cycle [68, 69]. Thus, PDGF, EGF, FGF and other factors may act to render cells competent for the action of IGF-1, which was termed progression factors. Muscle cells, chondrocytes, and osteoblasts grow rather well in the absence of any other growth factors when stimulated by IGF-1 [70], and, in semi viscous medium form colonies of highly differentiated cells [71, 72]. Osteoblasts increase type I procollagen messenger RNA levels under the influence of IGF-1 [73]. IGF-1 also stimulates the degree of differentiation of osteoblasts in newborn rats [74] and of primary embryonic chick muscle cells [70]. IGF-1 stimulates myofibril development in adult rat cardiomyocytes in vitro [75]. IGF-1 administered in vivo to hypoxic rats has the same effects as GH on chondrocyte differentiation in the epiphysis [76].

Insulin mimics the effects of IGF-1, and vice versa [77, 78]. In the case of the acute insulin-like effects on insulin target cells, insulin is always more potent than IGF-1 [77, 78]. Some of the insulin-like effects are mediated by a cross-reaction of the IGF-1 with the insulin receptor, but most are mediated by the IGF-1 receptor. Adipose tissue, heart muscle, and striated muscle, typical insulin target tissue, react to IGF-1 with increased glucose uptake [65, 77, 78, and 79]. IGF-1 usually increases glucose uptake to the same maximum as insulin, and depending on the tissue, are 5 to 100 times less potent than insulin [77]. In the rat heart, IGF-1 is about four to five times less potent than insulin in stimulating glucose uptake or 3-O-methyl glucose outflow [77]. IGF-1 stimulates glucose and amino acid uptake and increases glycogen synthesis of muscle in the same way as insulin and inhibits lipolysis of the fat cell in vitro [79]. These studies indicate that IGF-1 may be an important regulator of glucose utilization in vivo, either along with insulin or instead of insulin [79].

In DM, IGF-1, which is GH dependent, is decreased in the serum of diabetic animals and insulin dependent animals [80, 81, 82, and 83]. In diabetic swine, IGF-1 mRNA levels are decreased in heart, liver, and muscle, and this decreased gene expression is correlated with decreased serum levels [84]. There is evidence that insulin regulates serum IGF-1 levels by direct action on the liver and such that low insulin levels result in low serum IGF-1 levels [81]. And in turn, low serum IGF-1 levels have been associated with an up-regulation of IGF-1 receptors in certain tissues.

This study will investigate whether gingival tissue demonstrates that same up-regulation as evidenced in adipose tissue, heart muscle and striated muscle as well as other body tissues. The study will do so by examining immunohistochemically (IHC) the

presence of the IGF-1 receptor in human gingival tissue samples of diabetic patients versus controls, and determine if there is a difference in the expression in these two groups. An up regulation in the expression of IGF-1 receptor in the gingival tissue samples in diabetic patients versus controls would confirm my thesis.

## CHAPTER 2 MATERIALS AND METHODS

This study was conducted under the rules and regulations of the University of Florida Health Sciences Center Institutional Review Board asserting that all clinical investigative techniques, tissue management, and care was in concert with those expected and mandated with human use. This protocol was assigned the IRB # 622-20.

### **Patient Selection**

Gingival tissue samples from 30 of human patients were used for this study. Inclusion criteria were adult patients presenting to the University College of Dentistry at either the Graduate Periodontics Department or the Emergency Dental Clinic. All patients included in this study had presented for routine oral surgery or periodontal surgery and the tissue samples collected were gingival tissue normally excised and typically discarded as part of the standard of care in this type of treatment. Controls and diabetic patients were confirmed using a written health questionnaire and an oral interview. Individuals meeting the inclusion criteria were asked to participate in this study and given the informed consent form to sign. Informed consent was obtained prior to excision and collection of all tissues used. All of the test subjects had Type 2 diabetes mellitus.

### **Tissue Samples**

Gingival tissue samples were excised under local anesthetic (2% lidocaine with 1:100,000 epinephrine) during routine periodontal and oral surgery. The samples were immediately placed in 10% neutral buffered formalin v/v (pH 6.8-7.2 at 25 degrees

Celsius, Richard-Allen Scientific) and taken to the research laboratory. The tissue samples were then placed into a cassette and processed overnight in a Technicon. After processing they were embedded in paraffin. Serial histological sections of 6  $\mu\text{m}$  were obtained using a standard microtome and captured on glass slides from a warm water bath. A minimum of three slides were obtained from each tissue sample, with one stained with hematoxylin and eosin and at least 2 processed using an immunohistochemical technique. The slides were placed in a dry heat incubating oven for one hour at 110 degrees Celsius to remove the paraffin medium. Then the slides were placed and “cleared” in xylene for ten minutes to remove any residual paraffin. The slides were then carried through descending serial alcohol reagents by placing them in 100% alcohol, then 95% alcohol and then 80% alcohol to re-hydrate. Then the slides were washed with water. Then the slides were placed in antigen retrieval solution to clean the samples from enzymes. The slides were then placed in the dry heat incubating oven for thirty minutes at 110 degrees Celsius and allowed to cool for thirty minutes. All of the slides were then three times washed for fifteen minutes each with phosphate buffered saline (PBS) in order to remove all of the enzymatic digestion products. Immunohistochemistry requires that target retrieval (results in an increase in staining intensity with many primary antibodies) be performed to all formalin fixed paraffin embedded tissue sections mounted on glass slides. The goal is to eliminate all enzymes that may interfere with the intensity of the antibody staining.

### **Immunohistochemistry**

IHC staining techniques allow for the visualization of tissue (cell) antigens. These techniques are based on the immunoreactivity of antibodies and the chemical properties

of enzymes or enzyme complexes which react with colorless substrate-chromogens to produce a colored end product. Initial immuno-enzymatic stains utilized the direct method, which conjugated enzymes directly to an antibody with known antigenic specificity (primary antibody). Although this technique lacked the sensitivity of later methods, it allowed for the visualization of tissue antigens using a standard light microscope.

The sensitivity of IHC stains was significantly improved with the development of an indirect technique. In this method, enzyme-labelled secondary antibodies react with the antigen-bound primary antibody. A further increase in sensitivity of the indirect technique was achieved with the introduction of the peroxidase-antiperoxidase (PAP) enzyme complex [85]. In this method the secondary antibody serves as a linking antibody between the primary antibody and the PAP [85]. Subsequent developments in IHC exploited the strong affinity of avidin for biotin and resulted in the avidin-biotin complex (ABC) method [86]. The use of avidin-biotin interaction in immunoenzymatic techniques provides a simple and sensitive method to localize antigens in formalin-fixed tissues. Among the several staining procedures available, the ABC method, which involves an application of biotin-labeled secondary antibody followed by the addition of avidin-biotin-peroxidase complex, gives a superior result when compared to the unlabeled antibody method. The availability of biotin-binding sites in the complex is created by the incubation of a relative excess of avidin with biotin-labeled peroxidase. During formation of the complex, avidin acts as a bridge between biotin-labeled peroxidase molecules. The Biotin-labeled peroxidase molecules, which contain several biotin moieties, then serve as a link between the avidin molecules. Consequently, a "lattice" complex containing

several peroxidase molecules is likely formed. Binding of this complex to the biotin moieties associated with secondary antibody results in a high staining intensity [86]. The ABC method increased sensitivity when compared to the PAP method.

IHC was further improved with the labeled streptavidin biotin (LSAB) method which is based on a modified labeled avidin-biotin (LAB) technique. The LSAB method utilizes a biotinylated secondary complex with peroxidase-conjugated streptavidin molecules [87, 88]. In comparison to the ABC method, the LAB method and LSAB method have been reported to be four to eight times more sensitive [89, 90, 91]. The DAKO EnVision™ System, HRP two-step IHC staining technique was utilized in this study. The advantage of this system is that the protocol used is an extremely sensitive method and, as a result optimal dilutions of the primary antibody are up to twenty times higher than those used for the traditional PAP technique, and several-fold greater than those used for the traditional ABC or LSAB methods. This protocol offers an enhanced signal generating system for the detection of antigens in low concentrations or for low titer primary antibodies. Staining in this system is completed with 3,3 diaminobenzidine (DAB) substrate-chromogen which results in a brown colored precipitate at the antigen site.

A negative control was obtained by using a reagent which contained an antibody which exhibited no specific reactivity with human tissues or normal (non-immune) serum in the same matrix (solution) as the diluted primary antibody. The negative control reagent was the same subclass and animal species as the primary antibody, diluted to the same immunoglobulin as the diluted primary antibody using the same diluent. The

incubation period for the negative control reagent was the same as for the primary antibody.

The colored end-product of the staining reaction was alcohol insoluble and was used with an aqueous-based counterstain, DAKO Lillie's Modified Mayer's hematoxylin (code No. S3309). Counterstaining of the hematoxylin was followed with a thorough rinse in distilled water, and then the gingival tissue slides were immersed into a bath of 37mM ammonia as a bluing agent. Thirty-seven millimolar ammonia water was prepared by mixing 2.5mL of 15M (concentrated) ammonium hydroxide with 1 liter of water.

### **Reagents**

The monoclonal anti-human IGF-1 R antibody was supplied from R & D systems, Inc. (Minneapolis, MN), catalog number MAB391, clone 33255.111, lot number YY011031. The monoclonal anti-human IGF-1 R antibody was produced by a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse immunized with purified, insect cell line Sf 21-derived, recombinanthuman insulin-like growth factor 1 soluble receptor (rhIGF-1 sR). The IgG fraction of ascites fluid was purified by protein G affinity chromatography. The formulation was lyophilized from a 0.2  $\mu$ m filtered solution in phosphate-buffered saline (PBS). The endotoxin level was less than 10 ng per 1 mg of the antibody as determined by the LAL method. The antibody was reconstituted with sterile PBS to a concentration of 20  $\mu$ g/ml.

This antibody was selected for its ability to block cell surface human IGF-1 R mediated bioactivities induced by IGF-1 or IGF-2 and for use as a capture antibody in human IGF-1 R sandwich ELISAs. When used in combination with the biotinylated anti-human IGF-1 R detection antibody in sandwich ELISAs, less than 0.15% cross-reactivity

was observed with rhIGF-1, rhIGF-2, rhIL3 sR $\alpha$ , and rh TGF- $\beta$  sRII. The exact concentration of antibody required in order to neutralize the human cell surface.

IGF-1 R mediated bioactivity is dependent on the IGF-1 concentration and on the number and types of IGF-1 receptors present on the cell surface (a function of cell type and culture conditions). The Neutralization Dose for this antibody is defined as that concentration of antibody required to yield one-half maximal inhibition of the cell surface IGF-1 R mediated IGF response on a responsive cell line, at a specific IGF concentration. The Neutralization Dose for this lot of anti-human IGF-1 R antibody was determined to be approximately 0.025-0.075  $\mu$ g/ml in the presence of 6 ng/ml of rhIGF-2, using the human MCF-7 cell line.

The staining procedure steps were followed precisely as directed in the DAKO EnVision™ System, HRP, Universal, and Rabbit/Mouse (DAB) as follows:

#### Staining Procedure

##### STEP 1 - Peroxidase Blocking Reagent:

Excess buffer was tapped off the gingival tissue samples. A lintless tissue was used to carefully wipe around the gingival tissue sample to remove any remaining liquid and to keep reagent within the prescribed area. Peroxidase Blocking Reagent was applied to cover the gingival tissue sample. This was incubated for 5 minutes. Then the gingival tissue sample was rinsed gently with distilled water and placed in a fresh buffer bath.

##### STEP 2 - Primary Antibody or Negative Control Reagent:

Excess buffer was tapped off and slides were wiped off as in step 1. Enough primary antibody or negative control reagent were applied to cover the gingival tissue samples. The samples were then incubated for thirty minutes. The samples were then

rinsed gently with buffer solution from a wash bottle (the flow was not focused directly on the tissue) and the samples were then placed in a fresh buffer bath.

#### STEP 3 - Peroxidase Labelled Polymer:

Excess buffer was tapped off and the slides were wiped as in the previous steps. Labelled polymer was applied using enough to cover the entire gingival tissue samples. The samples were then incubated for thirty minutes and rinsed off as in step 2.

#### STEP 4 - Substrate-Chromogen:

Excess buffer was tapped off and the slides were wiped as in the previous steps. Enough of the prepared substrate-chromogen was applied to cover the entire gingival tissue samples. The slides were then incubated for ten minutes. The slides were then rinsed gently as in the previous steps.

#### STEP 5 - Hematoxylin Counterstain:

The slides were immersed in a bath of aqueous hematoxylin (DAKO Code No. S3309). The slides were then rinsed gently in a distilled water bath. The slides were dipped ten times into a bath of 37mM ammonia as a bluing agent. The slides were then rinsed in a bath of distilled water for five minutes.

#### STEP 6 - Mounting:

The gingival tissue samples were then mounted and coverslipped with an aqueous-based mounting medium (DAKO Glycergel® Mounting Medium, Code No. C0563).

### **Evaluating the Slides**

Each section of the gingival tissue samples was evaluated for the presence of intracellular brown DAB precipitate indicative of antibody binding. The staining intensity of anti-IGF-1 was assessed using the following evaluation; weak, moderate or

strong. The sum of the staining intensity was used for total immunoreactivity.

Immunoreactivity was scored on a scale of 0 to 4 as follows: 0 representing negative or less than 2% of positively stained cells, 1 representing 2-10% of positive cells, 2 for 11-25% positive cells, 3 for 26-50% positive cells, and 4 representing more than 50% positively stained cells. Dividing the total number of stained cells by the total number of cells present and multiplying this value by 100 provided the approximate percentage of positively stained cells [92, 93, and 94].

In general description terms, weak immunoreactivity refers to gingival tissue samples that had an average score between 0 and 1, moderate immunoreactivity to gingival tissue samples with a score of 2-3, and strong immunoreactivity to gingival tissue samples that had an average score between 3 and 4. Sections were examined and scored “blindly” under a light microscope by two previously calibrated investigators independent of each other [92, 93, and 94]. Dr. Indraneel Bhattacharyya of the Oral Pathology department and Dr. Matthew Rudolph of the Periodontics department were the grading investigators.

First positive slides were differentiated from the negative controls. Then three appropriate sections of each slide with adequate structural integrity were selected by the examiners and graded according to the previously described protocol. The sections were examined blindly by the two investigators independently of each other. As a group the slides were reviewed and each sample was assigned a grade.

## CHAPTER 3 RESULTS

To evaluate the staining intensity, only samples with similar structures were compared. A total of 22 samples with an adequate epithelium and connective tissue band out of the original 30 gingival tissue samples collected were evaluated and graded accordingly. Eight samples were eliminated due to inadequate structures for histological evaluation. Of the eight eliminated, seven were diabetic tissue samples and one was non-diabetic.

The negative control sections without the primary antibody did not stain with the dye (a negative staining result was achieved, see figure 3-1).

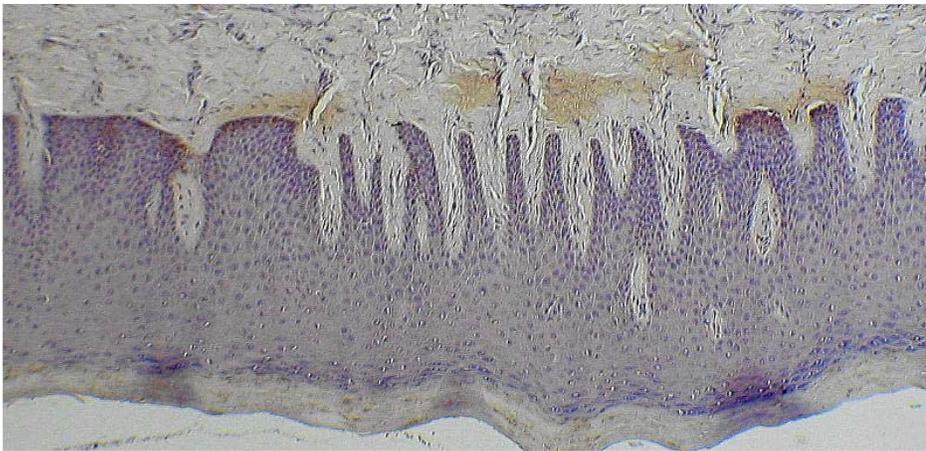


Figure 3-1: Negative Immunoreactivity

Four out of eight diabetic sections were all strongly positive (III). Two out of eight from diabetic patients was moderately stained (II) and two were weakly stained (I). Five out of fourteen from non-diabetic patients were strongly positive (III), three out of fourteen were moderately positive (II) and six were weakly stained (III) (Table 3-1). The

staining of the specimens was mainly concentrated in the cytoplasm of epithelial and endothelial cells.

All studied sections were stained positive for IGF-1 receptor (see figure 3-2).

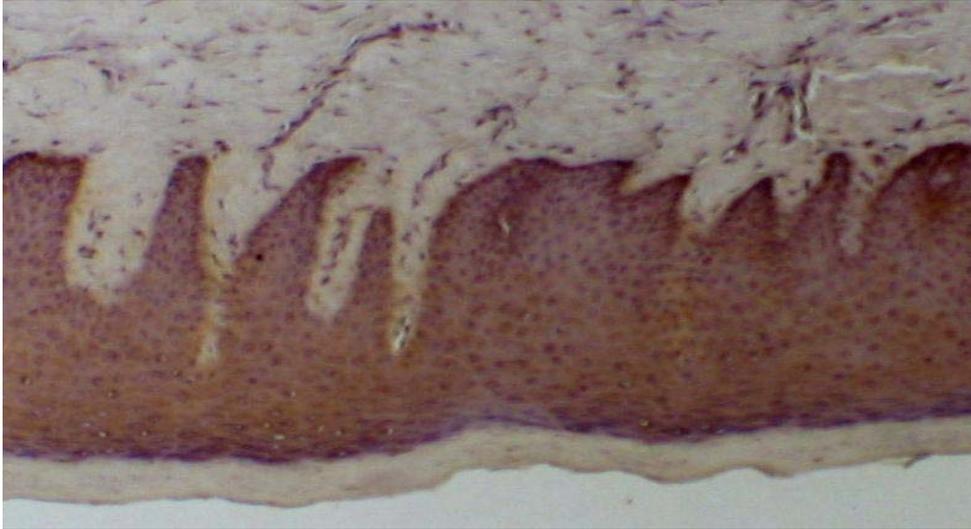


Figure 3-2: Positive Immunoreactivity

Table 3-1. Distribution of Data

|                     | Grade I | Grade II | Grade III | Total number of samples |
|---------------------|---------|----------|-----------|-------------------------|
| <b>Diabetic</b>     | 2       | 2        | 4         | 8                       |
| <b>Non-diabetic</b> | 6       | 3        | 5         | 14                      |

Total number of slides graded (n = 22)

Although there seems to be a trend for diabetic gingival tissue samples to have a greater (grade III) stain intensity distribution as well as a trend for non-diabetic gingival tissue samples to have a weaker (grade I) stain intensity (Table 3-2, Figure 3-3), there is no statistically significant difference (p value = 0.4) between the two groups. Statistical analysis was provided by Dr. Gary Stevens, University of Florida Biostatistics Department.

The values were attained using the Analysis for Linear Trend in Proportions.

Table 3-2: Percent Distribution

|                     | % of Grade I | % of Grade II | % of Grade III |
|---------------------|--------------|---------------|----------------|
| <b>Diabetic</b>     | 2/8 = 25%    | 2/8 = 25%     | 4/8 = 50%      |
| <b>Non-diabetic</b> | 6/14 = 42.9% | 3/14 = 21.4 % | 5/14 = 28.5%   |

Chi square for linear trend: 0.6516

p value: 0.4196

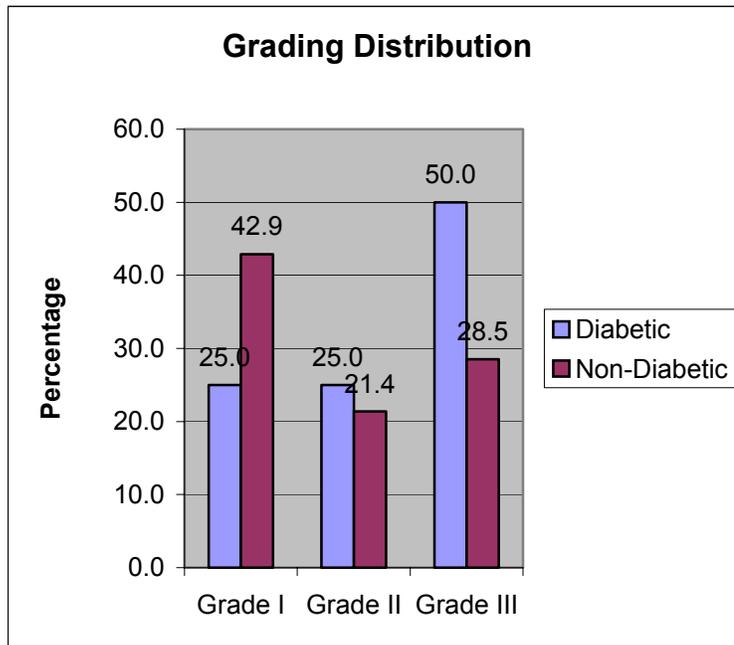


Figure 3-3: Grading Distribution

The universe of a study is the total collection of objects that are of interest in the project. For this investigation the universe is the collection of gingival tissue samples or more practically gingival tissue in adults. Each gingival tissue sample is the experimental unit. A convenience sample was used as the experimental units were chosen from those available in the local setting and agreed to participate. The response variable is the observation or outcome measurement that records the state of the physical phenomenon being studied. The response variable for this study is the observation of IGF-1 receptors (ranked grade I through III subjectively).

The population is the collection of response variable measurements on all experimental units in the universe. The collection of observations of IGF-1 receptors on each gingival tissue sample make up the population. The U-sample is the collection of experimental units included in the study and the P-sample is the collection of response variable measurements from the U-sample. In this report the U-Sample is the collection of gingival tissue samples in this study and the P-Sample is the expression of IGF-1 receptors on the human gingival tissue samples collected.

This is an observational study as it contains only observational factors. The response variable is ordinal as it can be ranked subjectively (grade I, II, or III). Ordinal response variables are the only type of response variable which do not have common distributions to determine mathematically. Hence, they are referred to as distribution free. The statistical analysis of ordinal response variables has historically not involved parameters. This has led to the development of a group on analyses generally referred to as nonparametric because they are not based on population parameters. Thereby validating the use of analysis for linear trend and proportions.

## CHAPTER 4 DISCUSSION

In the present study expression of the IGF-1 receptor has been revealed on gingival tissue samples of both self reported Type 2 diabetics with periodontal disease as well as gingival tissue samples of self reported healthy non-diabetic subjects with periodontal disease. Although statistical significance could not be established between both groups certain trends were apparent. A trend for increased expression of IGF-1 receptors in diabetics as well as a decreased expression in controls was obvious (see figure 3-3). These results are consistent with previous studies showing that the expression of the IGF-1 receptor is up-regulated in diabetes [95, 96, 97, and 98].

The role of the IGF system in the pathogenesis of diabetes and diabetic complications is speculative at this point in time. There is a growing body of evidence that for its role in microvascular complications and its ability to mediate the proliferative features of these complications. The tissue-specific nature of these complications in the context of a systemic metabolic disturbance suggests the possibility of autocrine or paracrine dysregulation which may be mediated at a number of levels: increased local IGF production, increased sequestration of circulating IGF by IGF binding proteins, or tissue specific increases in IGF receptor numbers [112].

Studies of circulating IGF-1 levels in diabetic patients for the most part have demonstrated decreased IGF-1 levels (50-90%) [113]. Although there have been some studies that showed normal or elevated levels [114]. The relationship between glycemic control and IGF-1 is also not quite definitive. Most studies have found an inverse

correlation between measures of metabolic control and plasma IGF-1 levels [113]. There have been others that have not found such a relationship, but they may have had insufficient power to detect a relationship or there may have been confounding factors [114]. Interventions which improve glycemic control have been shown to increase circulating IGF-1 levels [115]. The study of laboratory animals with streptozotocin (STZ) induced diabetes have supported the theory of reduced IGF-1 levels in diabetes and an inverse relationship with metabolic control [116].

The long term complications of diabetes include microangiopathy (retinopathy, nephropathy, neuropathy, and periodontal disease) and macroangiopathy which results in an increased incidence of cardiovascular disease [113]. Diabetes is associated with vascular smooth muscle cell and endothelial cell dysfunction [117]. Abnormalities include impairment of vasodilatory responses, increased levels of endothelium-derived von Willebrand's factor, and decreased levels of prostacyclin and plasminogen factor. Vascular basement membrane thickening and increased vascular permeability are present in diabetic patients and animals models [117]. Endothelial cells have IGF receptors and secrete IGF binding proteins. They are exposed to circulating IGFs and to IGFs synthesized by vascular smooth muscle cells [118]. IGFs have metabolic and trophic effects on endothelial cells and vascular smooth muscle cells [117]. A potential role of IGFs in the development of retinopathy and nephropathy as well as other tissues has been investigated. The specific role of IGFs in the development of periodontal disease associated with diabetes has not been investigated at this time.

IGF-1 binding to IGF-1 receptors and IGF-1 stimulated tyrosine kinase activity are unimpaired in red blood cells in type 2 diabetics [119]. IGF-1 receptor number and basal

and IGF-1 stimulated receptor  $\beta$ -subunit phosphorylation are increased in the placenta of type 1 diabetics with poor glycemic control [120, 121]. A number of diabetic patients have immunoprecipitating autoantibodies to IGF-1 receptors, some of which inhibit IGF-1 binding and may result in resistance to IGF-1 [122]. Patients with severe insulin resistance and insulin receptor antibodies (type B insulin resistance) have a higher incidence of IGF-1 receptor antibodies [122, 123]. All these studies indicate that there are tissue specific regulatory and compensatory actions that can be correlated with the type of diabetes that the patient has and the level of metabolic control that the patient is able to attain.

Type 1 DM is usually diagnosed in children and young adults, and was previously known as juvenile diabetes or insulin-dependent diabetes. In type 1 DM, the body does not produce insulin. Insulin is necessary for the body to be able to use glucose. Glucose is the basic energy source for the cells in the body, and insulin takes the glucose from the blood into the cells. Type 2 DM is the most common form of diabetes (90-95% of all cases). In type 2 DM, either the body does not produce enough insulin or the cells ignore the insulin. When glucose builds up in the blood instead of going into cells, it can cause problems. Right away cells in the body may be starved for energy. Over time, high blood glucose levels may hurt various organs and tissues including eyes, kidneys, nerves, heart and the oral cavity. The significance of this disease can not be overstated. Diabetes is the seventh leading cause of death in the United States. The total annual economic cost of diabetes in 2002 was estimated to be \$132 billion, or one out of every 10 health care dollars spent in the United States.

The causes of type 1 DM appear to be much different than those for type 2 DM, appearance of type 1 DM is suspected to follow exposure to an "environmental trigger," such as an unidentified virus, stimulating an immune attack against the beta cells of the pancreas (that produce insulin) in some genetically predisposed people. Risk factors for type 2 DM include older age, obesity, family history of diabetes, prior history of gestational diabetes, impaired glucose tolerance, physical inactivity, and race/ethnicity.

Both Type 1 and 2 DM are risk factors for periodontal disease. Patients with Type 1 DM, especially those that have had the condition for a long duration, have been found to have more gingivitis and more deep periodontal pockets than controls [15, 16, and 17]. Uncontrolled or poorly controlled diabetes has been shown to be associated with increased susceptibility to oral infections, including periodontitis [18, 19]. There have been several studies which have reported a significantly poorer periodontal health in Type 2 DM patients and some of these reports have provided epidemiologic parameter estimates of association and risk. The odds that have been reported for Type 2 diabetics to have greater risk of destructive periodontal disease are from 2.6 to 4.0 [20, 21, and 22]. There have also been two population-based surveys that have provided epidemiologic estimates of association for diabetes and attachment loss severity, with diabetic individuals being twice as likely to have more severe attachment loss as those without diabetes [23, 24].

Current evidence supports the fact that inferior glycemic control contributes to poorer periodontal health. Recent studies that have been published on the association between glycemic control and periodontal disease have shown that inadequate glycemic control is a significant factor associated with poorer periodontal health [25, 26, and 27].

The control of diabetes is directed at controlling the blood glucose levels within “normal limits”, and there is clear evidence that complications can be prevented by meticulous control of hyperglycemia [28, 29]. Monitoring the effectiveness of glycemic control is done by measuring the levels of glycated serum proteins, in particular glycated  $\alpha$ -hemoglobin (HbA1c), which because of its incorporation into the red blood cells gives an indication of the serum glucose levels over the preceding 2 to 3 months [30].

Impact of this study cannot be dismissed. Recently there has been attention developed concerning the use of recombinant human IGF-1 (rhIGF-I) in the treatment of Type 2 DM. Short term studies have demonstrated that rhIGF-1 increase insulin sensitivity leading to improved glycemic control and also have beneficial effects on lipid profiles [105, 106]. Furthermore free fatty acids are significantly reduced following acute or chronic rhIGF-1 administration [107]. The mechanism by which IGF-1 exerts these effects in vivo is unclear as IGF-1 can act through IGF-1 receptors, insulin receptors, or both [108]. If in fact up regulation proves to be evident in gingival tissue of diabetic patients, then perhaps some type of novel IGF-1 based local delivery system of therapeutic agent could be utilized to treat periodontal disease as well as controlling glycemic levels.

The theory that polypeptide growth factors such as IGF-1 and platelet-derived growth factor (PDGF) could be utilized to enhance regeneration of periodontal structures has been proposed previously. A review from 1987 discussed the properties of these natural biologic mediators to regulate the proliferation differentiation, motility and matrix synthesis of nearly all cell types [109]. The authors felt that these growth factors could facilitate and enhance periodontal regeneration by stimulating formation of mesenchymal

tissues including collagen, bone and cementum [109]. A preliminary study from 1989 reported initial observations following application of PDGF and IGF-1 to periodontitis-affected teeth in beagle dogs [110]. Growth factor treated sites exhibited significant amounts of new bone and cementum formation. A nearly continuous layer of osteoblasts lined the newly formed bone, and there was a dense cellular "front" at the coronal extent of the new bone. These preliminary results suggested that in vivo application of the combination of PDGF and IGF-1 may enhance regeneration of the periodontal structures. [110]

Another study compared bone promotion around dental implants which were augmented with ePTFE membranes alone or in combination with cortical demineralized freeze-dried bone (DFDB) or the combination of PDGF and PDGF/IGF-I [111]. Histologic measurements demonstrated that sites treated with ePTFE membranes plus PDGF/IGF-I had the highest bone density compared with sites which received ePTFE membranes alone or with ePTFE membranes and DFDB. The results of this study support the use of ePTFE membranes with PDG-F-BB/IGF-I as potential methods of promoting bone formation around dental implants [111].

Lack of statistical significance in this investigation can be explained due to several factors. One explanation for this is the study's small sample size. After careful review it was determined that a minimum of 100 samples from both diabetics and controls would be needed in order to obtain a statistically significant trend. The anonymity of sample collection as well as other IRB restrictions that hindered the ability to collect additional data that would aid in controlling for confounding factors (e.g. age, smoking, medications ), which may affect the expression of the receptor also affected the outcome

of this study. Most significantly was the restriction on the collection of blood samples and the inability to perform A1C tests on both diabetic and control patients to confirm the presence or absence of glycemic control. As previously discussed in this paper there is anywhere from 17-33% of the diabetic population that is not aware that they have diabetes. The A1C test shows glycemic control over a two to three month period prior to the test and is much more accurate than self reporting [7, 99].

Self reporting also may lead to underreporting of other significant systemic conditions. Dental patients routinely complete a medical questionnaire and have an oral interview during their routine care, but some patients may have undiagnosed systemic problems which can effect their dental treatment. An investigation in 1999 looked at thirty-nine consecutive patients referred for a periodontal evaluation who completed a written medical questionnaire and an oral interview [99]. They were referred to a hospital laboratory for a urinalysis, complete blood count, and a standard blood chemistry panel. The self-reported medical history responses were compared with the laboratory data and several abnormalities were noted. Abnormal levels were found with cholesterol, triglycerides, glucose, eosinophils, and monocytes. This study demonstrated that many patients are unaware of their current medical status and a significant number had undiagnosed abnormalities [99].

Despite its limitations, there were several reasons that the self reported health questionnaire was used in this study, as it is in many others. One of the main reasons is for convenience and cost. Another is the familiarity factor many patients have for self reported questionnaires. Many patients may not be willing to participate in a study if they feel that involvement in the study will require care outside the “normal” scope of

care. Dental patients do not routinely have blood drawn as part of the standard of care. Also it is important to note that other studies have found that even though the information provided by patients may not be as accurate as compared to laboratory testing, it is nevertheless a reliable source of information which can be utilized cost-effectively in research studies [100, 101].

The reliability and validity of using IHC analysis is another concern which must be addressed. Due to the subjective nature of this method there have been suggestions in the literature for ways in which to standardize this technique. One group has stated that reliable and precise quantitative IHC requires the use of control materials containing defined amounts of the target antigen and processed alongside the specimen combined with automated computer-assisted microspectrophotometry [102]. Use of this modality was beyond the scope of this investigation.

Another potential error in this study may be attributable to the subjective nature of the grading process. Interpretation of immunostains should be based on microanatomic distribution of the staining, proportion of positively stained cells, staining intensity, if relevant, and cutoff levels [102]. These parameters should be shown to be reasonably reproducible and should be clearly defined [102]. This was attempted by using the grading scale employed from previous investigations [92, 94]. The grading scale utilized in this study and many others are based on one that was first developed in 1968 [103]. This early grading scale was called the Chisholm-Mason's Scale and it and varying forms of this scale have been used for multiple IHC studies [92, 94].

But, due to its subjective nature many critics argue IHC analysis is not fully reproducible and lacks accuracy and validity. One review of the IHC technique stated that

“An ideal immunohistochemical screening panel would be one in which each antibody is 100% sensitive and specific for the target cell type (e.g., markers for epithelial neoplasms, lymphomas, sarcomas, etc.). Anyone who has practiced immunochemistry is well aware that this situation does not exist. High sensitivity is hindered by the loss of key antigens through formalin fixation and routine tissue processing” [104, p.59]. But this same author concluded that IHC provides for rapid and cost-effective diagnosis and that is why it is universally used for both clinical and academic applications.

The results of the present study should be viewed as preliminary due to the lack of statistical significance and other limitations discussed. As stated, the real importance of this study might lie in the actual presence of the IGF-1 receptor in gingival tissue. A finding that confirms what was previously only suspected. Further research in this area is needed to substantiate these findings. These future studies together with previous studies on this subject can guide treatment modalities that may limit or control co-morbid conditions associated with diabetes.

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## BIOGRAPHICAL SKETCH

Dr. Matthew Rudolph is originally from Fort Lee, New Jersey. After graduating from Fort Lee High School he went on to Tulane University in New Orleans, Louisiana, where he earned his bachelor's degree in biology. Then he attended the University of Pennsylvania School of Dental Medicine in Philadelphia where he earned his doctorate of dental medicine. While in dental school, Dr. Rudolph entered the United States Navy's Health Science Collegiate Program. After earning his DMD, he was commissioned as an officer in the United States Navy Dental Corps. In the Navy his duty stations have included Patuxent River Naval Air Station in Lexington Park, Maryland, the USS John F. Kennedy (CV 67) out of Mayport, Florida, the USS Frank Cable (AS 40) out of Agana, Guam, and the United States Naval Academy in Annapolis, Maryland. Currently Dr. Rudolph is serving full time out service at the University of Florida College of Dentistry as a second year resident in the graduate periodontics department. He is married to Kimberly Jones-Rudolph, also a dentist and an associate professor at UFCD, and they have three children. Dr. Rudolph's current research involves studying Insulin-like Growth Factor-1 receptor in the gingival tissues of diabetics and controls.