



## Assessment of Salivary Stress Marker (Peroxidase) Levels in Completely Edentulous Patients: crosssectional study

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### KEYWORDS

Innovation, completely edentulous, Implant, residual ridge resorption, peroxidase.

### ABSTRACT :

#### BACKGROUND :

Patients' diet quality and nutritional status decline as a result of their avoidance of hard meals, including both vegetables and non-vegetables, due to their reduced ability to chew due to tooth loss. Peroxidases are either directly or indirectly associated with several major human disorders, including cancer, periodontal disease, convulsive illnesses, Parkinson's disease, and coronary artery disease (CAD). These diseases can arise as a result of a variety of different chemicals that are involved in the metabolic pathways of peroxidase action, such as auto-antibodies, flavonoids, and thiocyanates. Recent research provides indirect support for the notion that dental implant therapy-based oral rehabilitation is beneficial for people with diabetes. In both diabetic and non-diabetic fully edentulous individuals, we assessed salivary glucose levels to see if the diabetic patient may have any detrimental effects on issues after surgery, implant failure, and peri-implantitis.

**AIM:** The present study aims to evaluate the peroxidase levels in completely edentulous patients.

**MATERIALS AND METHOD:** Add the sample and reagent into each well of the human lactoperoxidase ELISA kit. At 37 degrees, incubate for one hour. Rinse the plate five times. Combine solutions A and B for the substrate. Incubate at 37 degrees for ten minutes. Colour develops when a stop solution is added. Within ten minutes, determine the OD density value.

**RESULTS AND DISCUSSION:** According to the current investigation, there was a statistically significant difference in the groups' baseline means ( $p=0.00$ ). Patients with diabetes had a mean value of 3.09, and for those with diabetes, it was 2.77, both of which are statistically significant.

**CONCLUSION:** A significant increase was observed in peroxidase levels in the completely edentulous patients but an elaborate study needs to be conducted with a larger sample size.



## INTRODUCTION :

Deviations from normal protein, lipid, and carbohydrate metabolism are the hallmarks of diabetic mellitus (DM), a clinical disorder. These abnormalities cause a multitude of problems, including retinopathy, nephropathy, neuropathy, cardiovascular symptoms, etc. Dental issues, such as periodontal disease, dental caries, salivary dysfunction, dry mouth, diseases of the oral mucosa, and oral infections, are the most common symptoms among individuals with diabetes mellitus. Due to its tiny size and ease of diffusion through semipermeable membranes, saliva carries glucose, particularly in situations where blood sugar levels are elevated. Diabetic membranopathy is another reason why saliva contains glucose. Any modification to the blood vessel's basement membrane, according to Harrison and Bowen, may enhance the quantity of glucose that enters the saliva. In those with a history of diabetes, oral health issues are often associated with the metabolic management of the disease, and inadequate glucose regulation can have a detrimental effect on the salivary glands. Venepuncture is a frequent treatment used to measure blood sugar levels, but it can be unpleasant and upsetting, especially for small children. As a result, a different approach becomes necessary.

Peroxidase activity in a cell is regulated by a variety of various circumstances. Different pathogen kinds, such as bacteria, can potentially increase or decrease an organism's peroxidase mRNA levels<sup>1-3</sup>. Exposure to heavy metals such as Cd, Cu, Cr, and Ni either activates or inhibits the amount of peroxidase. The amount of selenium affects the GPx mRNA and its enzyme activity because glutathione peroxidases include selenocysteine, an important amino acid. Multiple methods have been proposed to explain why peroxidases are expressed more or less during certain disorders, however, these explanations fall short of fully describing the function of these enzymes<sup>4</sup>. For example, it has been discovered that certain tumour cells have decreased GPx function (GSH) because they reduce glutathione levels. This GSH shortage stimulates tyrosinase activity. Melanin is also regarded as an active oxygen species scavenger, hence tyrosinase activation in melanoma cells results in greater pigmentation as a compensation strategy for the lower GPx levels<sup>56</sup>.

The debilitating, permanent condition known as edentulism is referred to as the "ultimate marker of disease load for oral health". While complete tooth loss has become less common over the last ten years, edentulism is still a dangerous illness, particularly in the

elderly. Dentulism may coexist with functional and sensory abnormalities of the salivary glands, mouth muscles, and mucosa.<sup>7</sup>. It is anticipated that the edentulous population will have less tissue regeneration and resistance, which may jeopardize the oral mucosa's protective function. Studies have shown a connection between oral mucosal issues, ageing, and wearing dentures.<sup>8</sup> Angular cheilitis, oral candidosis, traumatic ulcers, and denture stomatitis—an inflammatory disease of the palatal mucosa common in people who wear complete dentures—are among these disorders. People who wear dentures are approximately three times more likely to develop angular cheilitis, stomatitis, and hyperplasia, according to MacEntee et al. These problems have the potential to expose an individual to both internal and external diseases, thus it is important to take into account their prevalence while evaluating the oral health of the elderly population.<sup>9</sup>. Aspiration pneumonia has not been directly linked to edentulism, but in sensitive individuals, a possible association between denture plaque and the illness has been explored.<sup>10</sup>.

Up until recently, it was believed that the only purpose of peroxidases, which are heme-containing enzymes, was to provide oxidative defence against pathogenic microorganisms such as bacteria that infiltrate immune cells and release large amounts of these enzymes at sites of injury and inflammation. These enzymes' roles in bone biology provide enormous promise as a treatment for bone regrowth. Inhibiting osteoclast differentiation and bone resorption (Panagopoulos et al., 2017b), driving angiogenesis (Panagopoulos et al., 2015), regulating collagen biosynthesis and matrix mineralization by human osteoblasts (DeNichilo et al., 2016), and regulating fibroblast collagen extracellular matrix biosynthesis (MPO and EPO) are examples of mammalian peroxidases and plant-derived peroxidase proteins, such as HRP and SBP. The process of repairing bones is closely linked to these biological mechanisms. The changes to the alveolar ridge that follow tooth extraction and continue long after the extraction socket has healed are referred to as "residual ridge resorption" (RRR). It is a difficult procedure that happens whether or not the patient has dentures on.<sup>11</sup>. According to research by Bergman and Carlsson, resorption differs across individuals and within the same individual at different times. Individuals with higher levels of bone resorption at first usually show higher levels of resorption over time.<sup>12,13</sup>. According to Lammie, pressure resorption of the ridge results from disuse



atrophy, which is the atrophying mucosa's search for a smaller area, if the patient does not wear a denture. Forces from the denture pressing against the ridge may cause the denture wearer to resorb. A free radical (FR) is any entity that possesses one or more unpaired electrons and can exist on its own. Free radicals cause tissue damage through a variety of mechanisms, including lipid peroxidation, protein oxidation, DNA oxidation, oxidation of essential enzymes, and activation of pro-inflammatory cytokines. Since RRR is a chronic inflammatory illness, tissue damage in the form of bone resorption may result from the free radicals that are generated. One of the mechanisms that might be implicated in lipid peroxidation.<sup>12</sup>. Our team's vast knowledge and research background have resulted in publications of the highest calibre.<sup>14-2324-29</sup>

## MATERIALS AND METHOD :

### Sample size calculation

A university hospital's prosthodontics department conducted a clinical trial. G Power software was used to determine the sample size, which included 60 participants (30 with diabetes and 30 without). Every patient who was chosen for the study was made aware of it and offered the option to voluntarily provide written consent.

### Method of sampling

Participants in the research had a primary complaint of having all of their teeth replaced. Study participants ranged in age from 40 to 65 and included 30 diabetes and 30 non-diabetic individuals who were receiving implant-supported total denture treatment. A straightforward random sample procedure was used [figure 1].

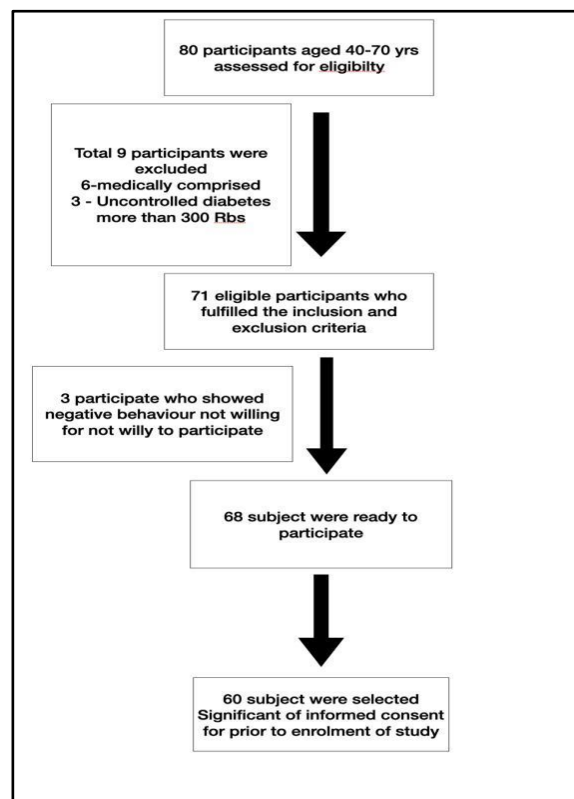
### Study design

Crosssectional study

### Ethical Approval:

The institutional ethics committee granted the ethical approval. IHEC/SDC/UG-1897/22/PROSTHO/633 is the number.

### Selection criteria



**FIG 1 :** Flow chart depicts the patient selection for the study

### Inclusion

Completely edentulous diabetic and diabetic patients were considered as inclusion criteria

### Exclusion

Uncontrollable diabetic patients and cancer patients were considered as exclusion criteria.

### Procedure

1.5 ml of unstimulated saliva samples were provided by patients at the hospitals and Saveetha Dental College. There is an age range of 50 to 90 years old. The glucose oxidase-peroxidase method of the ECO GLUCO KIT, which breaks down glucose into gluconic acid, hydrogen peroxide, and a test sample (Ab in the presence of glucose oxidase), was used to measure the levels. Hydrogen peroxide further reacts with phenol and 4 aminoantipyrine to form a red quinoneimine dye complex, with peroxidase acting as a catalyst. The amount of glucose in the sample is directly proportional to the total glucose in milligrams, which defines the colour intensity.

### Data Collection

Only 60 of a total sample of 70 patients who had complete edentulism over the course of a month



matched the inclusion criteria. An outside reviewer examined the data.

### Statistical Analysis

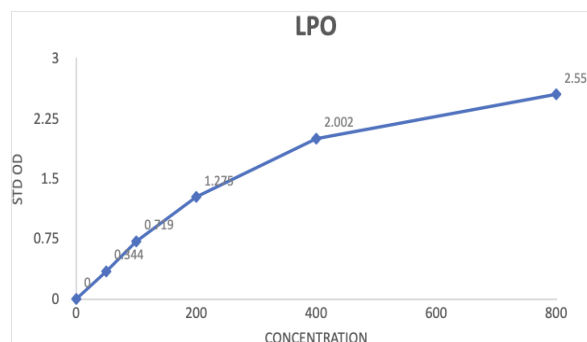
Before being exported and subjected to statistical analysis in SPSS (Statistical Package for Social Science for Windows Versions, 20.0), SPSS Inc. (Chicago IU, USA), data was imported into Microsoft Excel 2016 (Office 10). Following a statistical analysis with the Independent T-test ( $p < 0.05$ ), the data were tallied.



**Figure 2: Depicts Human peroxidase ELISA kit**

### RESULTS :

#### ASSAY – LPO



**Figure 3: Standard curve of LPO**

**TABLE - 2 INDEPENDENT T-TEST**

GROUP N	MEAN	STANDARD DEVIATION	SE	95% UPPER	95% LOWER	T VALUE	P VALUE
HEALTHY	30.00	3.09± 4.03	0.56	-31.48	-34.51	43.5	0.00*
DIABETIC	30.00	2.77±10.5	0.50	-31.48	-34.51	43.5	0.00*

### DISCUSSION:

Nowadays, there is increased interest in using saliva as a diagnostic fluid due to the capacity to swiftly and effectively assess all diagnostically significant steroids, antibodies, hormones, particular medications, and other compounds found in saliva. Saliva also has the advantage of being an easily collected and stored organic fluid. Because excited saliva may dilute and modify the PH, unstimulated saliva has been utilized instead. It has previously been proposed that the increased permeability of the basement membrane linked to insulin-dependent diabetes mellitus increases the amount of serum-derived components that leak through gingival fissures into the entire saliva. The semipermeable basement barrier is easily penetrated by the little glucose molecule. The increased levels of glucose in salivary secretions were attributed to the gingival crevicular fluid.

According to the current investigation, there was a statistically significant difference in the groups' baseline means ( $p = 0.00$ ). Nonetheless, the mean value for individuals with diabetes was 3.09, and it is statistically

significant for diabetic patients, who have a mean value of 2.77. According to earlier research, the majority of clinical trials found that diabetic patients could benefit from dental implants just as much as healthy persons. The inclusion of managed diabetics in nearly all research may seem to be the cause. The development of microvascular complications and, as a result, early or late implant failure are caused by continuous hyperglycemia.<sup>(30)</sup>

Various dangerous germs often enter the human body through the mouth route. Saliva acts as the body's first line of defence against these microorganisms, with salivary peroxidase (SPO) serving as the main peroxidase. Salivary peroxidase makes up about 80% of oral peroxidases, and MPO makes up the remaining 20%. SPO inhibits both Gram-positive and Gram-negative oral and extraoral bacteria. SPO also demonstrates antifungal and antiviral properties. Additionally, salivary peroxidase builds an oral antioxidant defence mechanism, especially against the attack of free radicals produced by cigarette smoke that may cause oral cancer.<sup>31,32</sup> It has been noted that





smoking even one cigarette causes a significant decrease in OPO activity. The salivary proteins have an increase in carbonylation as a result, which is a sign of protein oxidative damage. The OPO activity of heavy smokers is lowered in response to the damaging effects of hydroxyl radicals and thiocyanate ions produced by elevated H<sub>2</sub>O<sub>2</sub>. Through saliva-mediated processes, this makes oral cancer more likely to develop in heavy smokers.<sup>31,33</sup>

Since erythrocytes and other tissues contain GPx1, it is the most prevalent of the GPx family proteins. By coupling the oxidation of various hydrogen donors with oxyhemoglobin, it shields these cells from the damaging effects of H<sub>2</sub>O<sub>2</sub>. Numerous illnesses, including hyperglycemia, hyperbilirubinemia, and obesity, have been linked to the over- or under-expression of GPx1<sup>34</sup>. The gastrointestinal tract is where GPx2 is primarily expressed, and squamous cell carcinoma and colorectal cancer both increase its production. Additionally, it has been discovered that GPx3 is expressed greater during chemotherapy in obese, diabetic, and head and neck cancer patients. Additionally, endometrial cancer causes this enzyme to be downregulated<sup>35</sup>.

One of the three ways the body's defence system responds to free radicals is by destroying, scavenging, or eliminating them. An imbalance between the body's antioxidant defence system and the production of reactive oxygen species (ROS) or free radicals (FR) is what leads to oxidative stress. Biomarkers of the antioxidant defence system include enzymatic antioxidants such as glutathione peroxidase (GPx), catalase, and superoxide dismutase (SOD), as well as non-enzymatic antioxidants such as vitamin C, vitamin E, and uric acid.<sup>11,36</sup> The sort of process that results in tissue damage determines the biomarkers of free radicals. These biomarkers provide us with information about the nature and severity of the injury that resulted from the inflammatory phenomena, and they also indicate how far along the disease process. The antioxidant enzyme superoxide dismutase defends and keeps the cell healthy. Humans have three SOD isoenzymes: Cu/Zn SOD is present in the cytoplasm and nucleus, Mn-SOD is located in the mitochondria, and extracellular EC-SOD is discovered<sup>11,36,37</sup>. Its major job is to catalyse the dismutation of O<sub>2</sub> to H<sub>2</sub>O<sub>2</sub>, which removes harmful ROS from the cellular environment. Since lipid peroxidation is a result of oxidative stress, tissue damage manifests as bone resorption<sup>11</sup>. The primary byproduct of the lipid peroxidation process is

malondialdehyde (MDA). In this study, the levels of MDA and SOD in people who have lost all of their teeth are evaluated, and these levels are correlated with age, gender, the types of bone resorption, the duration of edentulism, and the effect of wearing dentures.<sup>38</sup>

When a toothless patient has more than 10 implants, peri-implantitis can affect all of the implants or only one implant in those without periodontitis. In our study, peri-implantitis affected 4.70 implants on average per patient. This circumstance did not affect the total amounts of MPO and MDA in the saliva. Our major goal was to determine whether peri-implantitis could increase total salivary levels of OS markers. Most published investigations measure the gingival sulcus of the affected implants.<sup>39</sup> This explains why the experimenters were able to obtain such high quantities. Thus, in patients with a mean of four implants, peri-implantitis can be understood as a localized, self-limited illness with no systemic consequences on OS and inflammatory response. Since the data pertain to the overall concentrations of MDA and MPO in saliva, they cannot be exclusively attributed to a specific implant or tooth. According to studies like the ones by Linkman and colleagues that looked at the total concentration of OS biomarkers in saliva, patients with peri-implantitis had higher MPO levels.<sup>39-41</sup> The MPO concentration in the peri-implant sulcus is measured in the remaining studies, and the results show levels that are higher than those found in healthy implants. As a lipid oxidation marker, MDA is the most important and studied molecule; nevertheless, no prior study has measured MDA concentration in patients with peri-implantitis. MDA is a byproduct of the peroxidation of polyunsaturated fatty acids. Nevertheless, significant amounts of MDA were found in the crevicular fluid of periodontal teeth when it was studied in individuals with periodontal disease<sup>39,40</sup>. Recent research provides indirect support for the notion that dental implant therapy-based oral rehabilitation is beneficial for people with diabetes. Chew-intensive foods are avoided by patients who have lost teeth, which can result in inadequate nutritional status and incorrect metabolic management. The patient can enhance nutrition and metabolic management with adequate oral rehabilitation.<sup>42</sup> However, it is yet unknown how the effectiveness of dental implants is impacted by the standard of diabetes care and the severity of the illness.<sup>43</sup> It is essential to be able to predict outcomes during dental implant surgery to control risk properly. To determine if the diabetic patient may have any negative effects on problems following surgery, implant



failure, and peri-implantitis, we performed a salivary glucose level examination on both diabetes and non-diabetic edentulous patients.

## CONCLUSION :

Different oxyradical forms, which were formerly thought to be detrimental byproducts, are now understood to carry out several crucial cellular tasks. However, any imbalance in their production results in a variety of illnesses, placing additional strain on peroxidases. Peroxidases actively participate in hormone production, oxyradical oxidation, and innate immunity in addition to playing a vital role in the antioxidant defense system of living things. Different peroxidases have specificities for organs, tissues, cells, or sub-cells and are either directly or indirectly linked to some human disorders. The expression of peroxidases either rises or falls throughout various disorders. Their different manifestations during pathological conditions are explained by several processes. Although peroxidases have been used as risk markers for many different human diseases, their ideal purpose is still unknown.

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