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Comparative Assessment of Biomarker (ESM-1) For Evaluating Inflammation and Endothelial Cell Responses in Peri-Implantitis Patients with and Without Diabetes Along with Related Success of Implant Prosthesis: An Original Research Study

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KEYWORDS

Endothelial Cell-Specific Molecule-1 (ESM-1), Biomarker, Inflammation, Peri-Implantitis, Periodontology, Peri-Implant Crevicular Fluid, ELISA

ABSTRACT:

Aim: This study aims to assess endothelial cell-specific molecule-1 (esm-1) as a biomarker for evaluating inflammation and endothelial cell responses in patients with and without diabetes, as well as those suffering from peri-implantitis.

Materials and Methods: The initial cohort consisted of 50 patients, of which 40 were deemed suitable for implant placement after a thorough clinical examination. Informed consent was obtained from all participants, and detailed cone beam computed tomography (CBCT) assessments identified any skeletal anomalies that might affect implant success. The surgical procedure included local anaesthesia, careful incisions, and osteotomy to prepare the implant site. After a two-month healing period, a gingival former facilitated soft tissue contouring before the prosthetic phase began three months post-surgery. Biomarkers for esm-1 in peri-implant crevicular fluid (PICF) samples were analyzed using enzyme-linked immunosorbent assay (ELISA) after collection and freezing. The study included four groups for comparison: Group 1 (10 healthy individuals missing a first molar), Group 2 (10 with peri-implantitis), Group 3 (10 diabetic individuals), and Group 4 (10 diabetic individuals with peri-implantitis).

Statistical Analysis and Results: The study involved 40 patients (27 males, 13 females) missing a mandibular first molar and seeking dental implants. Participants were divided into four groups. Group 1 included 10 healthy individuals, where most showed no significant changes in bone loss, probing depth, or inflammation. Endothelial cell-specific molecule-1 (esm-1) was detected in Peri-Implant Crevicular Fluid (PICF) using ELISA. Group 2 comprised 10 patients with peri-implantitis, revealing two cases of bone loss, three with probing depth issues, and two with inflammation. Group 3, consisting of 10 diabetic patients without peri-implantitis, showed two cases of bone loss, two with probing depth concerns, and two with inflammation. Group 4 included 10 diabetic patients with peri-implantitis, experiencing more complications: three with bone loss, four with probing depth problems, and three with inflammation.

Conclusion: This study found that diabetic patients with peri-implantitis (Group D) had higher ESM-1 levels, indicating a stronger inflammatory response. ESM-1 is an effective non-invasive biomarker for assessing peri-implantitis severity. Elevated ESM-1 in the peri-implant crevicular fluid (PICF) suggests it could serve as an early indicator of worsening inflammation related to

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peri-implant conditions.

Introduction

Diabetes mellitus is a multifaceted and chronic metabolic disorder characterised by significantly elevated levels of blood glucose, also known as hyperglycemia. This condition stems from either inadequate insulin production by the pancreas or the body's inability to effectively utilise insulin, both of which lead to energy deficiencies and a range of severe complications.[1,2] Long-term complications of diabetes can be devastating, often resulting in serious health issues such as peripheral neuropathy, which causes damage to the nerves in the extremities, retinopathy, a leading cause of blindness affecting millions worldwide and nephropathy, which can progress to chronic kidney disease or renal failure. Diabetes is primarily classified into three main types.[3,4] Type 1 Diabetes (T1D) is considered an autoimmune disorder. In this type, the immune system erroneously targets and destroys insulin-producing beta cells in the pancreas, leading to an absolute deficiency of insulin. Individuals with T1D require lifelong insulin therapy to manage their blood glucose levels. This form of diabetes often manifests in childhood or adolescence, though it can develop at any age. Type 2 Diabetes (T2D) is the more prevalent form of diabetes, typically developing in adults.[5,6] It is characterised by insulin resistance, where the body's cells become less responsive to insulin, accompanied by a gradual decline in pancreatic insulin production. Risk factors for type 2 diabetes (T2D) include obesity, a sedentary lifestyle, increasing age, and genetic susceptibility. Unlike T1D, T2D can often be managed through lifestyle modifications such as diet and exercise, though some patients may also require oral medications or insulin therapy as the disease progresses. Gestational Diabetesoccurs during pregnancy when hormonal changes contribute to elevated blood glucose levels. This condition usually resolves after childbirth, but it significantly raises the mother's risk of developing Type 2 diabetes later in life. Monitoring and managing blood sugar levels during pregnancy are crucial for the health of both the mother and the baby.[6,7] Common symptoms of diabetes include increased thirst (polydipsia), frequent urination (polyuria), constant fatigue, and blurred vision. Managing diabetes effectively requires a comprehensive approach that encompasses dietary changes, regular physical activity, adherence to prescribed medications, and, when necessary, insulin therapy. Additionally, diabetes is closely linked to increased risks and severity of oral health problems, particularly periodontal (gum) disease. Poor gum health can exacerbate blood glucose control, creating a vicious cycle that necessitates integrated medical and dental care. Furthermore, individuals with diabetes often have a compromised immune system, making them more vulnerable to infections and leading to complications such as bone loss and tooth loss.[8,9] Biomarkers serve as essential quantifiable indicators within the body, offering critical insights into both the presence of diseases and the body's response to various treatments. These biomarkers encompass a wide spectrum of measurements, ranging from straightforward assessments like blood pressure and glucose levels to more complex evaluations, such as genetic analyses. Among the most significant types of biomarkers are those related to endothelial cell (EC) responses, which include a variety of adhesion molecules and inflammatory mediators. These specific biomarkers are pivotal in providing a deeper understanding of the body's physiological state, particularly in relation to inflammation, coagulation processes, and various functional changes. They play a crucial role in elucidating conditions such as cardiovascular diseases, systemic infections, and cancers, revealing the intricate biological mechanisms at play and aiding in the development of targeted therapeutic strategies.[10,11] These biomarkers reflect changes in cellular activity and can significantly enhance the assessment of patient health and the development of personalised treatment strategies. Overall, a thorough understanding of the intricacies of diabetes and the role of biomarkers not only facilitates more tailored medical care but also promotes a holistic approach to managing this pervasive condition, ultimately improving patient outcomes and quality of life.[12,13] This study aims to assess endothelial cell-specific molecule-1 (esm-1) as a biomarker for evaluating inflammation and endothelial cell responses in patients with and without diabetes, as well as those suffering from peri-implantitis.

Materials and Methods

This study was planned, designed and executed in the department of Periodontology of the institute. This study was aimed to investigate the implications of endothelial cell-specific molecule-1 (esm-1) as a biomarker for assessing inflammatory responses and endothelial cell activity in patients missing a mandibular first molar and seeking implant-supported solutions. The initial cohort comprised 50 patients. A thorough clinical examination was conducted, resulting in the identification of 40 patients deemed suitable candidates for implant placement, all of whom

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expressed interest in receiving an implant-supported prosthesis. The demographic profile of this population included adults aged between 35 and 60 years, encompassing a balanced representation of both male and female participants. The group included individuals with diabetes as well as non-diabetics. To maintain the integrity of the study, patients exhibiting mental instabilities or systemic diseases were explicitly excluded from the research, thereby ensuring a more homogeneous sample that would facilitate clearer results. Prior to the initiation of any treatment procedures, informed consent was obtained from all study participants, ensuring ethical compliance. To evaluate the participants' oral health status and to identify any skeletal anomalies, detailed cone beam computed tomography (CBCT) analyses were carried out. These imaging assessments were particularly focused on detecting severe bony defects, especially in the region corresponding to the mandibular right first molar, which could impact the success of the implant placement. To mitigate the risk of postoperative infections a common concern in surgical procedures, all patients were instructed to perform pre-operative mouth rinses with chlorhexidine mouthwash. A 0.2% chlorhexidine rinse was used for 1 minute before implant surgery to reduce bacteria in the mouth. 0.2% chlorhexidine rinse helps lower bacterial contamination during procedures, particularly when bone debris is created, which can cause infection. The surgical protocol began with the administration of local anaesthesia, precisely utilising lidocaine with epinephrine at a concentration ratio of 1:100,000. Following the establishment of effective anaesthesia, careful incisions were made, and an osteotomy was conducted to access the designated implant site. The preparation of the implant bed was executed with meticulous attention to detail, employing standard surgical protocol and chilled saline to create optimal conditions for osseointegration. A single operator highly trained and experienced in these procedures performed the implant placement to ensure consistency and reliability across the findings; the same implant kit was used for all participants. After an appropriate healing period of two months, a gingival former was utilized to promote ideal soft tissue contouring around the implant site. At the three-month interval following the placement of the crown, biomarkers for ESM-1 were analyzed in peri-implant crevicular fluid (PICF) samples. PICF analysis identifies early, subclinical metabolic and biochemical changes in peri-implant tissue, allowing for intervention before significant bone loss occurs. The collection process involved using precisely calibrated microcapillary pipettes to guarantee accuracy; samples were secured with cotton rolls to maintain sterility during their transfer to a phosphate-buffered solution. To ensure the integrity of the biochemical data collected, the samples were subsequently frozen at -70°C. Any contaminated samples were discarded to preserve the data's integrity. The levels of esm-1 in the samples were quantitatively assessed using enzyme-linked immunosorbent assay (ELISA) test kits, a method known for its precision in biomarker quantification. The study was structured into four distinct groups to facilitate comparative analysis. Group 1 consisted of 10 healthy individuals who were missing a mandibular first molar. Group 2 included another 10 individuals diagnosed with peri-implantitis, also presenting with a missing mandibular first molar. Group 3 was formed of 10 individuals diagnosed with diabetes who were similarly missing the mandibular first molar. Finally, Group 4 comprised 10 diabetic patients suffering from peri-implantitis and missing the same tooth. The primary aim of this comprehensive study is to thoroughly assess the viability of endothelial cell-specific molecule-1 (ESM-1) as a significant biomarker for evaluating inflammatory responses and endothelial cell activity in a diverse patient population, including those with and without diabetes and in individuals presenting with peri-implantitis. This exploration may yield critical insights into the relationship between biomarker presence and the clinical outcomes of implant-supported prostheses.

Statistical Analysis

In this study, we used SPSS software version 29.0 for all statistical analyses. To assess our findings, we employed the chi-square test to examine differences in proportions across groups. This method enabled a rigorous comparison of categorical data, ensuring our results accurately reflect trends and relationships in the dataset.

Results

This study involves a cohort of 40 patients, comprising both males and females, who presented with the absence of a mandibular first molar and expressed the desire for dental implant placement along with an implant-supported prosthesis. Table 1 provides a comprehensive statistical overview of the age and gender distribution of these contributing patients, while Graph 1 visually represents the demographic distribution and associated characteristics, illustrating a breakdown of 27 males and 13 females within the cohort. The participants were categorised into four distinct groups. Group 1 comprised 10 healthy individuals who were missing a mandibular first molar. Group 2 included 10 individuals diagnosed with peri-implantitis, along with the absence of a mandibular first molar. Group 3 consisted of 10 diabetic patients who

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also presented with a missing mandibular first molar but without any signs of peri-implantitis. Lastly, Group 4 was constituted by 10 diabetic individuals who had both peri-implantitis and a missing mandibular first molar. Table 2 details the findings for Group 1 (N=10), which focuses on healthy individuals missing their mandibular first molars. It also highlights the presence of endothelial cell-specific molecule-1 (ESM-1) in the Peri-Implant Crevicular Fluid (PICF), utilizing the Enzyme-Linked Immunosorbent Assay (ELISA) method for detection. Subsequent statistical analysis was performed using the Pearson Chi-Square test to ascertain the significance of the results. Within this group, bone loss, probing depth, and inflammation were reported in 1 patient for each category, while 7 individuals exhibited no significant changes. Table 3 presents the findings for Group 2 (N=10), which focuses on patients experiencing peri-implantitis with missing mandibular first molars. Similar to Group 1, the detection of ESM-1 was conducted via the ELISA method in PICF. The Pearson Chi-Square test was again employed for statistical analysis. In this group, bone loss, probing depth, and inflammation were observed in 2, 3, and 2 patients respectively, while 3 individuals showed no changes related to peri-implantitis. Table 4 showcases the findings for Group 3 (N=10), which consists of diabetic patients without peri-implantitis, all of whom are missing their mandibular first molars. The presence of ESM-1 was detected in PICF using the ELISA method. Statistical significance was evaluated using the Pearson Chi-Square test. Among these patients, 2 exhibited bone loss, 2 had probing depth issues, and 2 showed signs of inflammation, with a notable 4 patients presenting no changes. Table 5 elaborates on Group 4 (N=10), which includes diabetic patients who have been diagnosed with peri-implantitis alongside their missing mandibular first molars. The ELISA method was employed here as well to determine the presence of esm-1 in PICF. The results were similarly assessed using the Pearson Chi-Square test. In this group, bone loss, probing depth, and inflammation were noted in 3, 4, and 3 patients respectively, and all patients exhibited no changes associated with diabetic peri-implantitis. Lastly, Table 6 provides a summary of the estimations across all studied groups using one-way ANOVA analysis, which enables a comparative evaluation of the results across the different patient categories. This comprehensive statistical approach enhances our understanding of the relationships and potential implications of these variables in the context of missing mandibular first molars and associated conditions.

Table 1: Age & gender based statistical description of contributing patients

Age Group (Yrs)	Male	Female	Total	P value
35-40	7	3	10	0.08
41-45	8	2	10	0.30
46-50	4	4	8	0.01*
51-55	5	2	7	0.60
56-60	3	2	5	0.50
Total	27	13	40	*Significant
*p<0.05 significant				

Graph 1: Patients demographic distribution and associated details

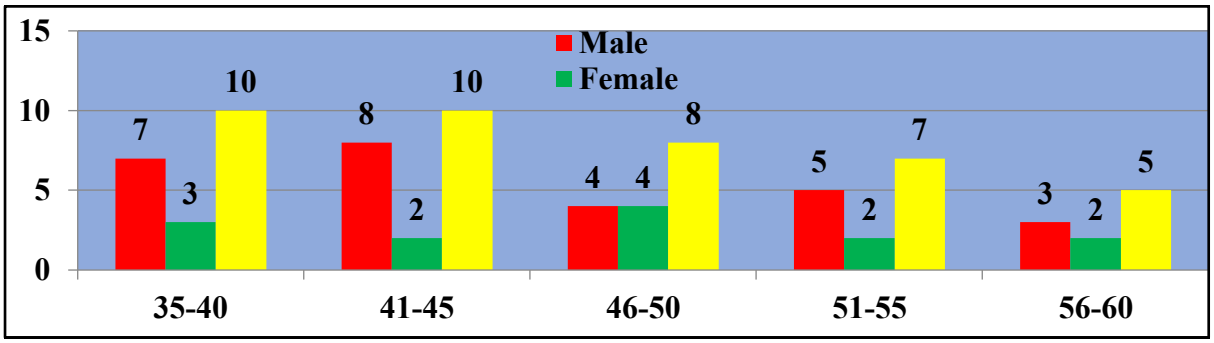

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Table 2: Group1 (N=10) Healthy individuals with missing mandibular first molars and the presence of endothelial cell-specific molecule-1 (ESM-1) using the Enzyme-Linked Immunosorbent Assay (ELISA) method in Peri-Implant Crevicular Fluid (PICF). Statistical analysis was then conducted using the Pearson Chi-Square test to determine the significance of the results

Evaluation Criteria	N	Mean	Std. Dev.	Std. Error	95% CI	Pearson Chi-Square Value	df	p value
Bone Loss	1	1.03	1.01	1.02	1.02	1.09	1.08	0.01*
Probing Depth	1	1.03	1.01	1.02	1.02	1.09	1.08	0.01*
Inflammation	1	1.03	1.01	1.02	1.02	1.09	1.08	0.01*
No Change	7	1.18	2.27	2.26	2.35	2.37	2.46	1.0
*p<0.05 significant								

Table 3: Group 2 (N=10) patients with peri-implantitiswith missing mandibular first molars and the presence of endothelial cell-specific molecule-1 (ESM-1) using the Enzyme-Linked Immunosorbent Assay (ELISA) method in Peri-Implant Crevicular Fluid (PICF). Statistical analysis was then conducted using the Pearson Chi-Square test to determine the significance of the results

Evaluation Criteria	N	Mean	Std. Dev.	Std. Error	95% CI	Pearson Chi-Square Value	df	p value
Bone Loss	2	1.09	1.08	1.12	1.08	1.19	1.108	0.02*
Probing Depth	3	1.10	1.09	1.15	1.19	1.20	1.18	0.06
Inflammation	2	1.09	1.08	1.12	1.08	1.19	1.108	0.02*
No Change	3	1.10	1.09	1.15	1.19	1.20	1.18	0.06
*p<0.05 significant								

Table 4: Group 3 (N=10) Diabetes patients without peri-implantitis with missing mandibular first molars and the presence of endothelial cell-specific molecule-1 (ESM-1) using the Enzyme-Linked Immunosorbent Assay (ELISA) method in Peri-Implant Crevicular Fluid (PICF). Statistical analysis was then conducted using the Pearson Chi-Square test to determine the significance of the results

Evaluation Criteria	N	Mean	Std. Dev.	Std. Error	95% CI	Pearson Chi-Square Value	df	p value
Bone Loss	2	1.09	1.08	1.12	1.08	1.19	1.108	0.02*
Probing Depth	2	1.09	1.08	1.12	1.08	1.19	1.108	0.02*
Inflammation	2	1.09	1.08	1.12	1.08	1.19	1.108	0.02*
No Change	4	1.12	1.19	1.20	1.22	1.26	1.19	0.07
*p<0.05 significant								

Table 5: Group 4 (N=10) Diabetes patients with peri-implantitis with missing mandibular first molars and the presence of endothelial cell-specific molecule-1 (ESM-1) using the Enzyme-Linked Immunosorbent Assay (ELISA) method in

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Peri-Implant Crevicular Fluid (PICF). Statistical analysis was then conducted using the Pearson Chi-Square test to determine the significance of the results

Evaluation Criteria	N	Mean	Std. Dev.	Std. Error	95% CI	Pearson Chi-Square Value	df	p value
Bone Loss	3	1.10	1.09	1.15	1.19	1.20	1.18	0.06
Probing Depth	4	1.12	1.19	1.20	1.22	1.26	1.19	0.07
Inflammation	3	1.10	1.09	1.15	1.19	1.20	1.18	0.06
No Change	0	-	-	-	-	-	-	-
*p<0.05 significant								

Table 6: Estimation amongst all studied groups using one-way ANOVA

Variables	Degree of Freedom	Sum of Squares ∑	Mean Sum of Squares m∑	F	Level of Sig. (p)
Between Groups	6	2.981	2.427	1.2	0.01*
Within Groups	17	2.246	2.562	_	
Cumulative	217.22	781.12	*p<0.05 significant		

Discussion

Wadivkar P et al. reviewed in their study that Diabetes mellitus (DM) is a multifaceted metabolic disorder characterised by chronically elevated blood glucose levels, which can lead to an array of long-term complications affecting various organ systems. This condition encompasses several types, the most prominent being type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM). T1DM is predominantly an autoimmune disease that often arises during childhood or adolescence, resulting in the body's immune system attacking and destroying the insulin-producing beta cells in the pancreas. Without sufficient insulin production, individuals with T1DM are unable to regulate their blood glucose levels effectively, necessitating lifelong external insulin administration.[14,15] Lin X et al included in their study that, in contrast, T2DM primarily emerges in adults, particularly those who are middle-aged or older, and is closely linked to lifestyle factors such as obesity, a sedentary lifestyle, and suboptimal dietary choices that foster insulin resistance. Over time, the pancreas may struggle to produce enough insulin to overcome this resistance, leading to persistently high blood sugar levels and various complications. One of the serious complications associated with diabetes is periodontitis, a chronic inflammatory condition that adversely affects the structures supporting the teeth, including the gums and bone.[16,17] Guthrie RA et al included in their study that Research indicates that individuals with diabetes experience a markedly higher prevalence of severe periodontitis, with studies showing that approximately 59.6% of diabetic patients are diagnosed compared to only 39% of non-diabetic individuals. The oral health consequences for those with diabetes can be profound, as they typically endure more extensive tissue damage, presenting with significant attachment loss of the gums to the teeth, accelerated alveolar bone loss, increased incidence of bleeding during dental procedures, and a heightened risk of tooth mobility and eventual loss. These dental issues not only diminish the quality of life but also act as a potential marker for poor metabolic control, highlighting the necessity for a comprehensive and integrative dental care strategy tailored to the needs of diabetic patients.[18,19] Brooks-Worrell B et al included in their study that biomarkers serve as essential tools in the diagnosis and management of diseases, providing objective and quantifiable measures that enable healthcare professionals to assess disease progression and monitor treatment efficacy effectively. In the case of diabetes, particularly type 2 diabetes mellitus

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(T2DM), pivotal metrics such as blood glucose levels and HbA1c (a blood test that indicates the average blood glucose levels over the preceding two to three months) are indispensable for evaluating glycemic control and guiding therapeutic decisions.[20,21] Schwarz F et al showed in their study that beyond these standard measurements, more complex genetic and biochemical markers have emerged as significant indicators of T2DM. Among these, interleukin-6 (IL-6) and C-reactive protein (CRP) have garnered particular attention. IL-6 is a pro-inflammatory cytokine secreted by a variety of cell types, including adipose tissue cells (adipocytes) and immune cells such as macrophages. It plays a crucial role in orchestrating the body's inflammatory response and contributes to the development of insulin resistance, a hallmark feature of T2DM.In parallel, CRP is a protein synthesised by the liver in response to inflammation and serves as a reliable biomarker for systemic inflammation. Elevated levels of CRP are often indicative of chronic inflammatory states, which are commonly associated with various conditions, including diabetes. Together, these biomarkers not only enhance our understanding of the underlying pathophysiology of T2DM but also provide valuable insights into individual patient risks and potential treatment pathways.[22,23] Allinson JL et al included in their study that furthermore, the endothelium, a monolayer of endothelial cells that lines the inner walls of blood vessels, plays a vital role in maintaining vascular homeostasis and regulating a myriad of processes, including hemostasis, inflammation, and vascular tone. Endothelial dysfunction is both a contributing factor to and a possible consequence of numerous diseases, particularly diabetes and cardiovascular conditions. In diabetes, endothelial dysfunction can lead to increased vascular permeability, chronic inflammation, and thrombosis, significantly complicating the management of diabetic patients while raising their risk for cardiovascular events. Understanding the intricate interconnections between diabetes and endothelial health is essential for developing effective therapeutic approaches aimed at enhancing patient outcomes and minimising complications associated with this chronic condition.[24,25]

Conclusion

In the context of their research limitations, the authors concluded that patients in the diabetes group suffering from peri-implantitis (designated as Group D) exhibited significantly elevated levels of ESM-1. This increase implies a compounded inflammatory response within endothelial cells, indicating a more pronounced reaction in these patients. Moreover, ESM-1 emerges as a highly effective and non-invasive biomarker for gauging the severity of peri-implantitis. The presence of diabetes appears to amplify both systemic and local inflammatory processes, resulting in higher baseline levels of ESM-1 in the peri-implant crevicular fluid (PICF) even before the onset of severe clinical symptoms associated with peri-implantitis. This suggests that increased ESM-1 could serve as an early warning signal for the severity of inflammation linked to peri-implant conditions, particularly in diabetic patients.

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