

Analysis of GLS1 Gene Expression Levels in Stage 2 Grade B Periodontitis Patients with and without Type 2 Diabetes Mellitus: An Ex-vivo Study

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ABSTRACT

Introduction: Diabetes Mellitus (DM), a metabolic condition marked by hyperglycaemia and periodontitis, a chronic inflammatory disease of the tissues supporting the teeth, are linked by similar pathophysiological pathways. Chronic hyperglycaemia deteriorates glycaemic control by weakening the host immune response and raising the risk of periodontal disease. Conversely, periodontal inflammation aggravates systemic inflammation and insulin resistance. Since Glutaminase 1 (GLS1) is associated with the inflammatory processes in both diseases, the present study investigates the expression levels of GLS1 in patients with diabetes and periodontitis to establish a potential pathophysiological link between the two diseases.

Aim: To assess and compare subgingival GLS1 gene expression among healthy individuals, patients with periodontitis and patients with both periodontitis and type 2 DM.

Materials and Methods: A ex-vivo study was conducted at Department of Periodontics, Saveetha Dental College, Saveetha Institute of Technical and Medical Sciences, Saveetha University, Chennai, Tamil Nadu, India, from June 2024 to July 2024. A total of 30 participants aged over 18 years with Stage 2 grade B periodontitis and stable glycaemic control {Glycated Haemoglobin (HbA1c)<7} were categorised into three groups with n=10 each: healthy controls, patients with periodontitis and patients with both periodontitis and DM. Patients with other co-morbidities were excluded. Clinical parameters, including Probing Pocket Depth (PPD), Clinical Attachment Level (CAL)

and HbA1c levels, were assessed. Gingival tissue samples were collected and analysed for GLS1 expression using quantitative real-time Polymerase Chain Reaction (qRT-PCR). Demographic and clinical data, including age, gender, glycaemic control and periodontal status, were also collected. Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) Statistics 27.0 (IBM Corp.) and GraphPad Prism 7.0 (GraphPad Software). One-way Analysis of Variance (ANOVA) and post-hoc tests were utilised for intragroup and intergroup comparisons, with a significance level set at $p<0.05$.

Results: The periodontitis and diabetes group had a significantly lower mean age compared to healthy controls ($p<0.05$), with no significant difference in gender distribution across groups ($p>0.05$). Clinical parameters (PPD, CAL, HbA1c) were significantly higher in the periodontitis and diabetic groups ($p<0.05$). GLS1 expression was highest in the periodontitis group and significantly differed from both the healthy and diabetic groups ($p<0.001$), while no difference was observed between the healthy and diabetic groups ($p=0.9369$).

Conclusion: The GLS1 expression was elevated in periodontitis patients but reduced in those with both periodontitis and DM, suggesting its potential as a biomarker influenced by systemic diseases. GLS1 may serve as a potential biomarker and therapeutic target, offering new avenues for integrated management of these chronic conditions. Further research is needed to fully elucidate the molecular mechanisms of GLS1 in the bidirectional relationship between periodontitis and DM.

Keywords: Biomarker, Gene therapy Glutaminase 1, Host response, Hyperglycaemia, Periodontal diseases

INTRODUCTION

Periodontitis is a chronic inflammatory condition that affects the gingiva, cementum, alveolar bone and periodontal ligament, which support teeth. It can eventually result in tooth loss and impaired oral function [1,2]. Chronic hyperglycaemia is a hallmark of Diabetes Mellitus (DM), a complicated metabolic disease that impacts several organ systems, most notably the micro- and macrovasculature and is caused by abnormalities in insulin secretion or action [3].

An increasing amount of research indicates that diabetes and periodontitis share a two-way relationship and are caused by similar pathophysiological processes. Hyperglycaemia weakens the host's immune system, making individuals more vulnerable to inflammation and periodontal infections. On the other hand, a periodontal infection exacerbates insulin resistance and poor glycaemic control by causing systemic inflammation [4]. People with diabetes are more likely to be affected by periodontitis and treating periodontal disease may help with glycaemic control [5-8]. The significance of investigating the

molecular connections between these two disorders is highlighted by these findings.

Glutaminase 1 (GLS1) is a crucial mediator in both disorders, according to recent research [9-12]. While GLS1 overexpression in inflammatory periodontal tissues points to a role in metabolic reprogramming during inflammation, GLS1 dysregulation is linked to insulin resistance and beta-cell dysfunction in diabetes [9-11]. GLS1 contributes to tissue injury by promoting the synthesis of proinflammatory mediators and meeting the increased energy requirements of immune cells. In animal models of both illnesses, GLS1 inhibition has shown therapeutic potential in lowering inflammation and enhancing glycaemic control [13-16].

Given its role in both diabetes and periodontitis, the present study aimed to evaluate and compare subgingival GLS1 expression levels in periodontitis patients with and without type 2 DM, as well as in healthy individuals, to elucidate its potential role as a central inflammatory mediator in the pathophysiological link between these two conditions.

MATERIALS AND METHODS

The present ex-vivo study was conducted at Department of Periodontics, Saveetha Dental College, Saveetha Institute of Technical and Medical Sciences, Saveetha University, Chennai, Tamil Nadu, India, between April 2024 and July 2024. Informed consent was obtained from patients. This study was approved by the Institutional Human Ethical Committee with IHEC reference number: IHEC/SDC/PERIO-2202/24/022. The collected samples were subjected to simultaneous assessment of GLS1 gene expression levels.

Sample size calculation: Sample size determination was performed using G power software Version 3.1.9.4, with standard effect size, a power of 80% and an alpha error of 0.05. Gingival tissue samples were collected from healthy individuals and patients diagnosed with periodontitis and/or DM.

Inclusion criteria: Participants aged over 18 years with Stage 2 grade B periodontitis [17], with probing pocket depth (PPD) ≥ 4 mm and clinical attachment level (CAL) ≥ 2 mm, stable glycaemic control (HbA1c < 7), who were under medication for DM and had no history of systemic inflammatory or autoimmune diseases, such as cardiovascular diseases, systemic lupus erythematosus, or rheumatoid arthritis, were included.

Participants with clinically healthy gingiva, with PPD ≤ 3 mm and CAL ≤ 2 mm and no DM (normoglycaemic individuals), were grouped under healthy controls.

Exclusion criteria: Pregnant women and lactating female patients, patients who have recently taken antibiotics or anti-inflammatory medication, individuals under long-term intake of immunosuppressants such as cyclosporine, tacrolimus, methotrexate, dapsone, hydroxychloroquine and steroids such as prednisone, methylprednisolone, or dexamethasone and smokers were excluded.

Study Procedure

Clinical and demographic data collection: Detailed demographic and clinical data were collected, including age, gender, glycaemic control (HbA1c levels) and clinical parameters (probing depth, clinical attachment level) [Table/Fig-1]. Other relevant medical history was also obtained.

Gingival tissue samples were collected from 30 participants categorised into three groups:

- Group 1: Healthy gingiva
- Group 2: Periodontitis
- Group 3: DM with periodontitis

Parameters	Group 1 (n=10)	Group 2 (n=10)	Group 3 (n=10)	p-value
Age (years)	44.14 $\pm$ 3.65	37.15 $\pm$ 2.55	36.05 $\pm$ 2.15	<0.001*
Gender (Male/Female)	3/7	6/4	7/3	0.18
PPD (mm)	2.25 $\pm$ 0.6	5.10 $\pm$ 2.25	5.45 $\pm$ 2.06	<0.001*
CAL (mm)	1.04 $\pm$ 0.5	2.1 $\pm$ 0.8	2.8 $\pm$ 0.4	<0.001*
HbA1C levels (%)	5.9	6.0	6.7	<0.001*

[Table/Fig-1]: Demographic and clinical details of study participants.

The samples were collected using sterile Gracey curettes. To preserve Ribonucleic Acid (RNA) integrity for subsequent gene expression analysis, tissue samples from the gingiva were introduced into RNA tubes immediately after collection [Table/Fig-2a,b].

The RNA Extraction and complementary Deoxyribonucleic Acid (cDNA) Synthesis: RNA extraction was performed on tissue samples from all three groups using Total RNA Isolation (TRIzol) reagent (Thermo Fisher Scientific, Waltham, MA, USA), followed by quantification and quality assessment using spectrophotometry and gel electrophoresis. The synthesis of complementary DNA



[Table/Fig-2]: (a) Sample collection from gingival tissue. (b) Collected samples.

(cDNA) was accomplished using the Takara 1st strand cDNA reverse transcription kits (Takara Bio, Inc., Kusatsu, Shiga, Japan), which facilitate the conversion of RNA into stable cDNA templates suitable for downstream gene expression analysis.

Quantitative Real-Time PCR (qRT-PCR): Quantitative real-time PCR (qRT-PCR) was employed to quantify GLS1 gene expression levels, utilising specific primers designed for GLS1 and reference genes such as Glyceraldehyde-3-phosphate Dehydrogenase (GAPDH), following established sequences [18]. A Biochemical-Radiochemical Laboratories (Bio-Rad) CFX1000 Real-Time PCR System from Bio-Rad Laboratories in Hercules, California, USA, was used for the procedure. According to the protocol, there was a 5-minute first stage at 95°C, followed by 40 cycles of 95°C for 10 seconds and 58°C for 30 seconds. The Comparative Threshold cycle ($2^{-\Delta\Delta CT}$) method was used to examine the results, which were expressed as relative mRNA levels, with GAPDH acting as the reference gene [19]. The Bio-Rad CFX Maestro 1.0 software (version 4.0.2325.0418) was used to automatically calculate gene expression in fold change.

In-Silico analysis: Protein interaction networks were investigated using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database (<https://string-db.org/>) [20] and the GLS1 gene's interaction network was analysed using GeneMania (<http://genemania.org/>) [21]. Using its associated network genes and proteins, Metascape (<https://metascape.org/>) was used to perform functional pathway analysis for GLS1.

STATISTICAL ANALYSIS

The SPSS Statistics 27 (IBM Corp.) and GraphPad Prism 7.0 (GraphPad Software) were used to conduct statistical analysis. Statistical significance was designated as a p-value of less than 0.05. One-way ANOVA and post-hoc tests were employed to compare the groups.

RESULTS

GLS1 Expression Levels

In the present study, the mean GLS1 gene expression levels were compared across three groups: healthy individuals, patients with periodontitis and patients with both diabetes and periodontitis [Table/Fig-3]. The periodontitis group exhibited the highest mean GLS1 expression (2.061 $\pm$ 1.14), followed by the healthy group (0.999 $\pm$ 1.06), while the diabetes with periodontitis group showed the lowest expression levels (0.916 $\pm$ 0.083).

Groups	Mean $\pm$ SD
Healthy (n=10)	0.999 $\pm$ 1.06
Diabetes with Periodontitis (n=10)	0.916 $\pm$ 0.083
Periodontitis (n=10)	2.061 $\pm$ 1.14

[Table/Fig-3]: Comparison of GLS1 gene levels of three groups.

Post-hoc analysis [Table/Fig-4] revealed a statistically significant increase in GLS1 expression in the periodontitis group compared to both the healthy group (p=0.0004) and the diabetes with periodontitis group (p=0.0002). However, no statistically significant difference

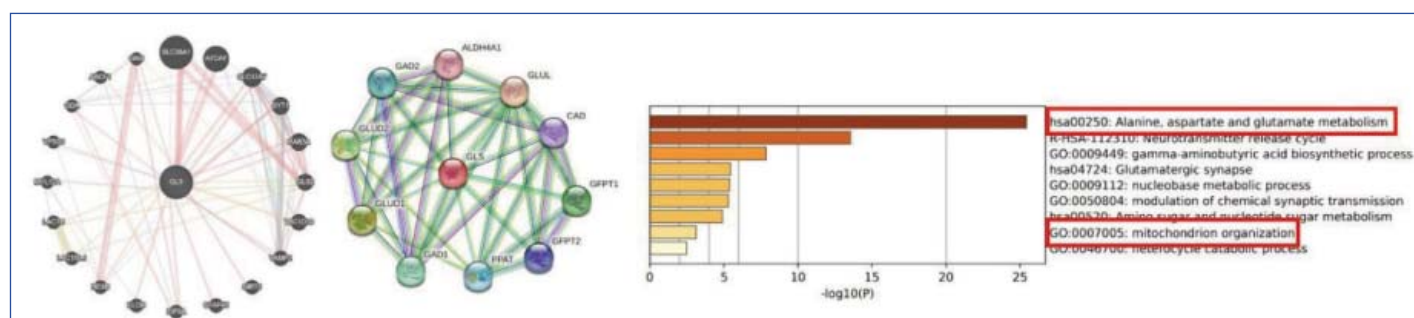
Groups	Mean difference	95% CI	p-value
Healthy (n=10) vs. Periodontitis (n=10)	-1.062	-0.464	0.0004*
Healthy (n=10) vs. Diabetes with Periodontitis (n=10)	0.083	0.680	0.9369
Periodontitis (n=10) vs. Diabetes with Periodontitis (n=10)	1.145	1.743	0.0002*

[Table/Fig-4]: Post-hoc analysis showing intergroup comparisons

was observed between the healthy group and the diabetes with periodontitis group ($p=0.9369$).

The network of genes and proteins interacting with GLS1 is depicted in [Table/Fig-5a], with edges showing interactions corresponding to different types of relationships (e.g., activation, inhibition). A more concentrated interaction network, depicted in [Table/Fig-5b], emphasises the role of GLS, which is essential for the metabolism of glutamate. The extensive connections imply that GLS1 plays a major role in glutamate-related metabolic pathways and may interact with several other proteins involved in related activities.

The bar graph in [Table/Fig-5c]: Functional Enrichment Analysis indicates the significance of various biological processes associated with GLS1. The highlighted pathways include:



Table/Fig-5: Gene (a) and protein (b) interaction network of GLS1. c). Functional enrichment analysis of GLS1 gene and protein interaction network highly associated with Alanine, aspartate and glutamate metabolism and mitochondrial organisation

- hsa00250: Alanine, aspartate and glutamate metabolism
- GO:0007005: Mitochondrion organisation

The $-\log_{10}(P)$ values suggest that GLS1 plays a crucial role in the metabolism of amino acids, especially those activities involving glutamate. Furthermore, the significant enrichment of “mitochondrion organisation” suggests that GLS1 may be involved in mitochondrial function and cellular energy dynamics.

DISCUSSION

The present study reveals differential expression of GLS1 among the study groups, with increased expression in patients with periodontitis and a relative decrease in those with both periodontitis and type 2 DM. This variation suggests that GLS1 may play a role in inflammatory and metabolic alterations associated with periodontal disease and that its expression could be modulated by systemic factors such as hyperglycaemia or antidiabetic medications.

The *GLS1* is a mitochondrial enzyme critical for converting glutamine to glutamate, fuelling the Tricarboxylic Acid Cycle (TCA) cycle and supporting the energy demands of activated immune cells. It also contributes to the synthesis of glutathione, a key antioxidant that regulates redox balance and protects against oxidative stress [3,22]. Dysregulation of GLS1 has been implicated in various metabolic conditions, including insulin resistance and obesity [9] and emerging evidence highlights its involvement in inflammatory responses.

In periodontitis, elevated GLS1 expression supports glutaminolysis, a metabolic shift associated with immune cell activation and the production of proinflammatory mediators such as cytokines and prostaglandins [10,11,23]. The present findings align with previous reports showing GLS1 upregulation in inflamed periodontal tissues

[13,14], further suggesting its role in the metabolic reprogramming characteristic of chronic inflammation.

In experimental models, inhibition of GLS1 activity has led to reduced periodontal inflammation, decreased bone resorption and improved clinical outcomes, reinforcing its therapeutic potential [13,14]. Interestingly, the decreased GLS1 expression observed in patients with both diabetes and periodontitis may reflect the complex metabolic interactions between these conditions.

Hyperglycaemia-induced oxidative stress, mitochondrial dysfunction and immune dysregulation are well-established in diabetes [24]. Additionally, inflammatory mediators from gingival tissues contribute to increased ROS production and compromised mitochondrial function, further aggravating tissue damage [25-27]. It is also plausible that pharmacological agents like metformin influence GLS1 activity. Prior studies have shown that metformin suppresses glutamine metabolism and reduces GLS1 activity in cancer cells without altering its expression levels [15,16]. This mechanism may extend to periodontal tissues, where metformin's anti-inflammatory properties have demonstrated clinical benefits, including when used as a local drug delivery agent [23].

Taken together, these findings support the potential of GLS1 as a biomarker for inflammation and a therapeutic target in managing

periodontitis, particularly in individuals with diabetes. However, the precise molecular mechanisms governing GLS1 regulation in the diabetic periodontal environment require further exploration. Future studies should investigate the influence of glycaemic control, medication profiles and genetic factors on GLS1 expression to better understand its diagnostic and therapeutic relevance.

Limitation(s)

Although informative, this ex-vivo study limits causal interpretation, highlighting the need for longitudinal research to clarify the relationship between GLS1 expression, diabetes and periodontitis.

CONCLUSION(S)

The GLS1 expression was found to be higher in individuals with periodontitis but lower in patients with both diabetes mellitus (DM) and periodontitis. These findings underscore the role of GLS1 in the pathophysiological interplay between periodontitis and type 2 DM, implicating it in inflammatory and metabolic reprogramming processes. The observed reduction in GLS1 expression among individuals with both conditions may be influenced by hyperglycaemia or antidiabetic medications such as metformin. These findings suggest that GLS1 holds potential as both a biomarker and a therapeutic target. Further studies are warranted to elucidate its underlying mechanisms and therapeutic applicability in managing these interrelated chronic diseases.

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