MATHEWS

Short Communication

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There is an Association between Type 2 Diabetes Mellitus and the Presence of HSV-DNA in a Subset of Oral Fibroepithelial Polyp Patients in Sri Lanka: Preliminary Findings Need Confirmation

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ABSTRACT

The relationship between Herpes Simplex Virus (HSV) and Type 2 Diabetes Mellitus (T2DM)remains uncertain. This study aimed to investigate the connection between type 2 diabetes and HSV positivity among male patients in Sri Lanka who present with oral fibroepithelial polyps. We collected samples of 25 fibroepithelial polyps patients from nine oro-maxillofacial units across six provinces in Sri Lanka. Tissue samples were taken from frozen excisional biopsies to prevent contamination and were tested for HSV-DNA using a real-time PCR assay. Data on sociodemographic information, clinical history, and current medical conditions were gathered through a pretested, interviewer-administered questionnaire. The results indicated that HSV-DNA was present in 44% of the samples, with 66.67% being HSV-1 and 33.33% being HSV-2. Interestingly, there was a statistically significant association between type 2 diabetes and the presence of herpes simplex viral DNA. The findings from this retrospective study warrant further investigation to validate the results and explore their implications in a larger sample size while controlling for confounding variables.

Keywords: Herpes Simplex Virus, Type 2 Diabetes, Hepertension, Periodontal Diseases, Inflammatory Metabolic Disease, Asymptomatic, Latency.

INTRODUCTION

Diabetes is one of the most common non-communicable, metabolic, and inflammatory diseases and a leading cause of morbidity, mortality, and reduced life expectancy globally [1]. Uncontrolled diabetes or higher blood glucose levels can promote not only retinopathy, neuropathy, and nephropathy. The risks of cardiovascular complications [2], hypertension, and end-stage renal failure [3]. Socio-demographic and lifestyle-related risk factors act individually for Type 2 diabetes mellitus (T2DM) demonstrating geographic and population specificity [4]. Epidemiologic evidence from South Asian countries has shown a progressive and alarming increase in the incidence of cardiovascular disease together

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with an increase in the prevalence of type 2 diabetes [5]. According to a recent systematic review and meta-analysis on the prevalence of (T2DM) and pre-diabetes in Sri Lanka, there has been a growing trend of diabetes and pre-diabetes over the last 30 years is alarming in Sri Lanka [6]. With the extensive application of molecular technologies to detect and quantify viral DNA, possible associations of HSV infection with T2DM [7,8].

Herpes simplex viral (HSV) infections may be symptomatic or asymptomatic. Benign mucosal oral lesions may mimic oral herpes [9]. Moreover, HSV infection seems ubiquitous with the global seroprevalence of 67% of HSV-1 and 13% of HSV-2 in 2016 [9] as cited by the same authors of this publication [10]. Primary infection leads to latent neural tissue eventually with the risk of severe acute necrotizing encephalitis for the rest of the lifetime. Researchers found not only that patients with diabetes (71.1%) had higher viral DNA detection rates than individuals without diabetes (30%) [11], but also that those who initially tested positive for HSV-2 were 59% more likely to develop prediabetes or diabetes compared to those who did not exhibit symptoms of a prior herpes infection resulted in the other study [12].

There is a lack of information on the association of type 2 diabetes, with the presence of HPV-DNA. In this background, this study aimed to findout the HPV -DNA status with type 2 diabetes, in a cohort of Sri Lankan male patients with oral FEPs.

MATERIAL AND METHODS

Ethical statement

The study sample of this study obtained ethical approval from the Faculty Research Committee, Faculty of Dental Sciences, University of Peradeniya, Sri Lanka (FRC/ FDS/ UOP/E/2014/32) and Griffith University Human Research Ethics Committee, Australia (DOH/18/14/ HREC), is a portion of representative subsample comprising 25 Sinhala males with histologically confirmed OSCC involving the buccal mucosa or tongue (cases) and 27 Sinhala males with FEP from the same anatomic sites of a large unmatched case-control study conducted in selected Oral and Maxillo-Facial (OMF) Units, located in 6 provinces namely, Western, Southern, Sabaragamuwa, North Western, Uva and Central in the Democratic Socialist Republic of Sri Lanka as described previously [13].

Sample size calculation

The present study examined a sample of 29 cases of oral squamous cell carcinoma (OSCC), compared to a control group of 26 FEP. This sample was part of a larger cohort that includes the majority of OSCC patients in Sri Lanka. The overall sample size was determined using the formula for unmatched case-control studies as provided by Kelsey et al. [14]. Therefore, the main sampleconsisted of 134 OSCC cases and 134 FEP controls, along with additional controls from other benign mucosal lesions, as described previously [13]. l studies. Consequently, the main sample included 134 OSCC cases and 134 FEP controls, along with additional controls from other benign mucosal lesions as described previously [13].

Patients and samples

Excissinal biopsies from Sinhala males aged ≥ 40yrs with Fibro-Epithelial Polyps in buccal mucosa or tongue and those who were not on antibiotics for the past two months [14].

Tissue sampling, genomic DNA extraction and quality assessment

Deep tissue samples (~100 mg each) were dissected from frozen (stored at -80°C) excisional biopsies to prevent contamination from the tumour surface. Genomic DNA was then extracted using the Gentra Puregene Tissue kit (Qiagen) according to the manufacturer's protocol for solid tissue [Cat no. 158689]. The quality assessment of the extracted genomic DNA was done using β-globin PCR with the primers PCO3 and PCO4 [13].

Real Time PCR for HSV-1 and HSV-2 DNA

The real time PCR assay for HSV-1 and HSV-2 were set up separately using primer sequences as standardized, validated and published previously [10].

Primers used for HSV rt PCR assay

HSV-1-wield-F CGGCGTGTGACACTATCG HSV-1-wied-R GGCGTGTGACACTATCG

Watz-HSV2-F CGCCAAATACGCCTTAGCA HSV2-R GAAGGTTCTTCCCGCGAAAT

Positive Controls: Extracted DNA from saliva from a patients known to be HSV-1and HSV -2 positive respectively.

Negative Controls: Extracted DNA from saliva from a patients known to be HSV-1and HSV -2 negative respectively.

Then, the real time PCR was performed on a Quant Studio 6- real -time machine with an initial step ofhold stage of polymerase activation step at 95^0 C for 5 minutes, followed by 45 cycles of amplication (5seconds denaturation at 95^0 C for 5 minutes; 30 seconds annealing (TM)/extension at 55^0 C) and melt curve stage of 3 steps (95^0 C for 10 minutes, 50^0 C for 10 minutes and 95^0 C for 15 minutes) 3. Overall run duration was 73 minutes and 24 seconds.

Data analysis

Amplicon detection was determined via rt PCR screen and melt curve analysis. Positivity was determined via qPCR screening and melt curve analysis. If the melt curve temperature does not equal that of the calibration curve, that sample is reported virus-negative. Data entering and analysis were performed by the SPSS-21, Statistical Package. The statistical significance of qualitative and quantitative data was obtained by descriptive statistics for percentage, frequency, mean, and standard deviation. Moreover, the Chi-Square test was used to assess the relationship between categorical variables, Fisher's exact for comparing groups (where cell counts were < 5), to findout the HPV –DNA positivity with type 2 diabetes, in a cohort of Sri Lankan male patients with oral FEP s.

RESULTS

This retrospective study was based on a subset of a group of oral fibroepithelial polyp patients in Sri Lanka. This subset consisted of 25 were chosen from a group of oral FEP patients in Sri Lanka to findout the differences between presence of HSV-DNA patients and absence of HSV-DNA with selected non-communicable diseases using statistics. Here we hypothesized that HSV positivity is associated with inflammatory non communicable diseases, that statistical difference between these two groups indicates an association of HSV positivity with T2DM.

Table 1. HSV status and type of HSV-DNA present in FEP subjects

HSV–DNA status	HSV-1	HSV-2 Total
In FEP	N %	N % N %
Positive	06 (66.7)	03 (33.3) 09 (36.0)
Negative	09 (56.3)	07 (43.7) 16 (64.0)
Total	**15 (60.0)**	**10 (40.0) 25** (100.0)

As depicted in Table 1, of 25 tissues, HSV-DNA was present in 9 (36.0%) and absent in 16 (64.0%) of the FEP tissue sample. Among the HSV-DNA positives, 6 (66.67%) and 3 (33.33%) were HSV-1 and HSV-2 respectively.

DISCUSSION

To the authors' knowledge, this study is the first to reveal an association between Type 2 Diabetes Mellitus (T2DM) and the herpes simplex virus (HSV) positivity in a subgroup of patients with oral fibroepithelial polyps in Sri Lanka. Consequently, there is a lack of scientific information to compare and contrast these nationally and internationally. In this study, we found a statistically significant association ($p<0.05$) between T2DM and HSV positivity. Our results align with previous findings [11], showing that HSV DNA in 10.4% of diabetic patients, and at least one type of viral DNA was found in 30% of individuals without diabetes. Among diabetic patients, Epstein-Barr virus (EBV) DNA most frequently (25.4%), followed by HPV (19.1%) and cytomegalovirus (CMV) at 5.2%. Additionally, a higher percentage of co-infection with EBV and HPV was observed among men (30.8%) [11].

Recent findings align with those of Pharm and colleagues in 2023 [15] which revealed that 11% of over 1,200 healthy participants in their latest trial tested positive for HSV-2-specific antibodies [15]. The trial required participants to maintain regular blood sugar levels. Additionally, the nondiabetic individuals were tested for seropositivity against other herpes viruses, including HSV-1, varicella-zoster virus (which causes chickenpox and shingles), Epstein–Barr virus, and cytomegalovirus (CMV). CMV was notably prevalent, with 46% of participants testing positive for antibodies, indicating a past infection [15]. Interestingly, after seven years, 364 participants developed prediabetes. Those who tested positive for HSV-2 at the start of the trial were 59% more likely to develop prediabetes or diabetes compared to those without evidence of past herpes infection. Our study reinforces the finding that individuals infected with herpes simplex virus type 2 (HSV-2) face a higher risk

of developing these conditions than those without such infections. Additionally, similar conclusions were made by European researchers regarding cytomegalovirus (CMV) [16]. However, establishing a direct link between type 2 diabetes and herpes viruses remains elusive [17].

However, diabetogenic viruses: enteroviruses, cytomegalovirus, hepatitis C virus, human immunodeficiency virus, severe acute respiratory syndrome coronavirus 2, among others, have been linked as triggers of type 1 (T1DM) and type 2 (T2DM) diabetes speculating this viral replication interfere with the signaling of hepatokinase molecules including GSK3 and AMP-activated protein kinase (AMPK) are impacted by the virus, negatively on β cellular reproduction, glucose metabolism, and metabolic dysregulation, though this field is yet poorly understood [18].

The understanding of the aetiopathogenesis of Type 2 Diabetes Mellitus (T2DM) has evolved, revealing a more complex picture than previously thought. It involves not only a decrease in insulin production and increased insulin resistance in the pancreas but also other factors related to fat deposition in the liver, which may further reduce insulin secretion. This lifestyle-related, non-communicable disease is now recognized as a metabolic inflammatory condition linked to increased susceptibility to infections due to impaired immune function [19].

T2DM is a well-known fact that diabetes is a disease even at the gestational stage. It can be considered a global pandemic, with 537 million diabetics and 6-7 million deaths due to diabetes and related complications latest data [20]. In the national context, the prevalence of type 2 diabetes in urban or rural areas may vary between 15-25% of the prevalence of type 2 diabetes in urban or rural areas [20]. Hence, the growing trend in diabetes and prediabetes over the last 30 years is alarming in Sri Lanka. Thus, the government of Sri Lanka needs to take steps to improve prevention, screening, diagnosis, and appropriate treatment of diabetes-associated bacterial, viral, and fungal infections due to impaired and overburdened immune systems emerged as a cause for concern with the widening of HSV and other members of Herpes viruses cross-talks in reactivation of life long existing viruses with latency either in peripheral neurons or B lymphocytes with the metabolism of high glycaemic index and processes starchy food and fizzy as well as sweetened drinks which hampers the remission of T2DM. Fortunately, this condition is reversible as dysregulated hepatokinase function due to HSV reactivation is not a lifelong condition. Production of a vaccine with efficacy and immunogenicity to eradicate HSV is the only answer to eradicate and eliminate all harmful effect of this herpes virus with the life long risk when infected. The small sample size is a limitation of this study.

CONCLUSION AND RECOMMENDATIONS

A significant association has been observed between type 2 diabetes mellitus (T2DM) and HSV positivity. Therefore, conducting more comprehensive case-control studies with larger sample sizes is strongly recommended. These studies should aim to account for potential confounding factors and verify the reliability of the findings from this research. The preliminary findings of this study should not be dismissed, as they provide novel and important insights to the scientific community. Furthermore, mechanistic studies are essential to help clinical scientists understand how viral proteins may influence insulin production in the beta cells of the pancreas. This process could potentially be reversed through the use of antiviral medications or by eliminating the virus using a vaccine with proven efficacy and immunogenicity.

AUTHOR CONTRIBUTIONS

Manosha Perera: Conceptualization; experimenta design; laboratory analysis; interpretation of results obtained by laboratory and statistical analysis; writing the original draft.

Irosha Perera: Conceptualization; study design; sample size calculation; performing excisional biopsies; followed patients and revision; statistical analysis; revision of the manuscript.

CONFLICT OF INTEREST STAEMENT

The authors declare no conflict of interest.

ETHICS STATEMENT

The microbiome profile of oral squamous cell carcinoma tissues in a group of Sri Lankan male patients which received ethical approval from the Faculty Research Committee, Faculty of Dental Sciences, University of Peradeniya, Sri Lanka (FRC/ FDS/UOP/E/2014/32) and Griffith University Human Research Ethics Committee, Australia (DOH/18/14/ HREC).

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DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

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