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Carriage Rate of *Streptococcus mutans* among Type II Diabetic Patients with Dental Caries and Compared with Non-Diabetic Population – A Comparative Study

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A B S T R A C T

The aim & objective of the study is to identify the Carriage rate of Streptococcus mutans from five anatomical surfaces of oral cavity of Type II Diabetes Mellitus and Non Diabetic population & to compare carriage rate *Streptococcus mutans* of Type II against Non Diabetic population. Swabs were collected from five different anatomical sites of the oral cavity from Type II and Non Diabetic patients of 30 each. Samples were confirmed for the species and then inoculated in Mitis Salivaris Agar to assess the Frequency of isolation and Colony Forming Units (CFU) of Streptococcus mutans. Observation of the study revealed that the Frequency of Isolation and Colony Forming Units were high in patients with Type II Diabetes Mellitus when compared to that of Non Diabetes Mellitus subjects.

Introduction

Diabetes Mellitus a major public health concern affecting approximately 2% of the population traditionally it classified as Type 1 and Type II [1&2]. Type-1 is referred to as Insulin Dependent Diabetes Mellitus and Type II as Non-Insulin Dependent Diabetes Mellitus with the archetypal signs and symptoms that includes the classic triad of frequent urination (polyuria), increased thirst (polydipsia), and increased hunger (polyphagia) and is also characterized by hyperglycemia due to insulin deficiency. Being it the most common endocrine disorder with multi factorial causes that is responsible for total lack of insulin which further progress to acute and chronic complications to set the disease process. It almost affects all the organ and tissues including the oral cavity. The oral cavity is harboured by both pathogenic and non pathogenic species that are well balanced and

habitat in different ecological niches such as on the tooth surfaces and shedding surfaces. According to the ecological plaque hypothesis certain factors exist, may be the systemic or local that triggers the shift in the proportion of the resident microbiota to a more or less pathogenic state and predisposes to disease process. One among the systemic cause is Diabetes Mellitus, which may influence certain changes affecting the oral micro biota especially pertaining to *Streptococcus mutans* that invariably alters and affects the soft and hard tissue of the oral cavity. Though there are different types of oral microbiota the study of *Streptococcus mutans* and their load and influence in the oral cavity in Type II Diabetes Mellitus needs more emphasis. Therefore the present study is intended to search the inter link for the role of *Streptococcus mutans* in Type II Diabetes Mellitus and thereby the study is aimed at the Frequency of isolation and the Colony forming units of *Streptococcus mutans* in various surface of the oral cavity and to correlate out the probable reason for the ecological change in Type II Diabetic patients.

To study the Carriage rate of *Streptococcus mutans* from the different anatomical site of the oral cavity of Type II Diabetes Mellitus patients and Non-Diabetes Mellitus population.
To compare Carriage rate of *Streptococcus mutans* of Type II Diabetes Mellitus patients with Non-Diabetes Mellitus population.

Materials and Methods

A comparative study was conducted from January 2014- May 2015.The study protocol has been approved by the Institutional Review and Institutional Ethics Board of Indira Gandhi Institute of Dental sciences, Sri Balaji Vidyapeeth (SBV). A total of 60 subjects participated in the study. The study population was divided into two categories as follows:

Group I: Type II Diabetes mellitus patients with Dental caries (30 samples).

Group II: Non-diabetes mellitus patients with Dental caries. (30 samples).

The subjects selected which met the following criteria:

Inclusion Criteria

1. Clinically proven Diabetes mellitus patients of Type II with Dental caries [Group I] formed the test groups.
2. Age and sex matched Non - Diabetes mellitus patients with Dental Caries [Group II] were included as controls to compare the results with test groups.

Exclusion Criteria

The following individuals / patients were excluded in our study.
1. Normal healthy individuals without Dental Caries.
2. Normal healthy individuals with other oral complaints.
3. Diabetic patients with other Oral/ Dental complaints except Dental Caries (Type II)

Swabs were separately collected from five anatomical sites such as Tongue dorsal surface, Tongue ventral surface, Buccal mucosa, Gingiva and Caries tooth surface of the Oral cavity. Initial confirmation for the presence of Gram positive cocci was done by Gram stain and viewed under microscope as shown [Photograph Number: 1]

The collected oral samples were serially diluted and 50 microliters of 1/1000 diluted samples (each) were subjected to anaerobic culture on Mitis Salivarius Agar (MSA) and incubated at 37 degree centigrade for 48 hours. Bacterial Colony forming units (CFU) was assessed by counting the typical *Streptococcus mutans* colonies and recorded for further statistical analysis. The colonies are recognised by their colony morphology based on raised, convex, undulate, opaque, pale-blue colonies that are granular (i.e., “frosted glass”) in appearance. [Photograph Number: 2]

Colonies may exhibit a glistening bubble on the surface due to excessive synthesis of glucan from sucrose. Colonies identified as *Streptococcus mutans* are further confirmed of their genus by Catalase negativity and by their hemolytic pattern (α and γ). [Photograph Number: 3, 4].

Results and Discussion

Out of 150 samples the frequency of isolation of S.mutans was 112 in Type II Diabetes mellitus [Group I], whereas the Frequency of Isolation of S.mutans was only 39samples in Non diabetic [Group II] and there was a significant difference between the Dorsal surface of Tongue ($p < 0.001$), Caries tooth surface ($p < 0.001$), Buccal mucosa ($p < 0.001$) & Gingiva ($p < 0.001$).

The Colony forming units of *Streptococcus mutans* was found to be more in Type II [Group I] compared to Non diabetic [Group II] in each of the five surfaces investigated following similar pattern as that of Frequency of isolation. The Colony forming units showed significant difference between the two given study groups in each of the surfaces investigated.

The Frequency of isolation & Colony forming units of *Streptococcus mutans* was highest in carious tooth followed by Gingiva in both the study groups. The *Streptococcus mutans* was isolated in all the samples from Caries tooth surface in each of the study group as expected. The Frequency of isolation and Colony forming units of *Streptococcus mutans* was the least from Ventral surface of tongue in the diabetic group patients, Type II [Group I].The Frequency of isolation & Colony forming units of *Streptococcus mutans* was the least from Buccal mucosa & Dorsal surface of Tongue respectively in Non diabetic [Group II]. *Streptococcus mutans* have been isolated on the surfaces where they are not generally isolated with more number colony yield. We know that if caries is not the factor, Diabetes Mellitus itself should be the influencing cause. The reason for this could be due to the difference in pathophysiology of disease in itself and in the way patient responds to insulin.

The oral cavity is stabilized by the resident flora, such as *Mutans Streptococcus* and *Streptococcus sanguis* that mostly seen on the teeth surfaces whereas *Streptococcus salivarius*, *Actinomycosis* and *Porphyromonas* habitat in the tongue, supra gingival and sub gingival areas respectively. In the mucosal surface the colonization of microorganism are relatively less and it dominated by the *Streptococcus salivaris and Streptococcus mitis* (Allcock *et al.,* 2001).

In our study the Colony Forming Units of *Streptococcus mutans* was evaluated in Group I, and Group II respectively and inferred that the mean and standard deviation for colony forming units of *Streptococcus mutans* was more in Type II [Group I] than Non diabetic [Group II] in each of the five surfaces investigated as in (Table.2).

The Colony Forming Unit in Dorsal surface of the Tongue, Buccal mucosa, Gingiva and in carious tooth surface, Ventral surface of the tongue showed statistically significant results with the value($p< 0.001$) between the groups. [Table number 3), (Table number 5) (Table. 6) (Table. 7) respectively.

Frequency Of Isolation and Colony Forming Units were comparatively more in [Group I] (Type II Diabetic Mellitus) and the results were concordant with study conducted by Sharm et al. (Alaluusua *et al.,* 1989) where there was Colony Forming Units for *Streptococcus mutans* was comparatively more in the Diabetic group than the nondiabetic group.

It is a known fact that one of the factor for the progression of dental caries is *Streptococcus mutans* and as in our study both the groups had caries we need to insist that the role of diabetes as the cause for the streptococcal count rather than the Dental caries.

a		Tongue Dorsal		
		Negative	Positive	
GROUP	I	18	12	$X^2 - 7.2$
	II	27	3	**p =0.007**

OR=6.0 [1.3 -31.52]

b		Tongue Ventral		
		Negative	Positive	$X^2 = 5.96$
GROUP	I	19	11	**p=0.013**
	II	27	3	

OR=5.21[1.12-27.5]

c		Buccal Mucosa		
		Negative	Positive	$X^{2=19.20}$
GROUP	I	12	18	**P=0.0001**
	II	28	2	

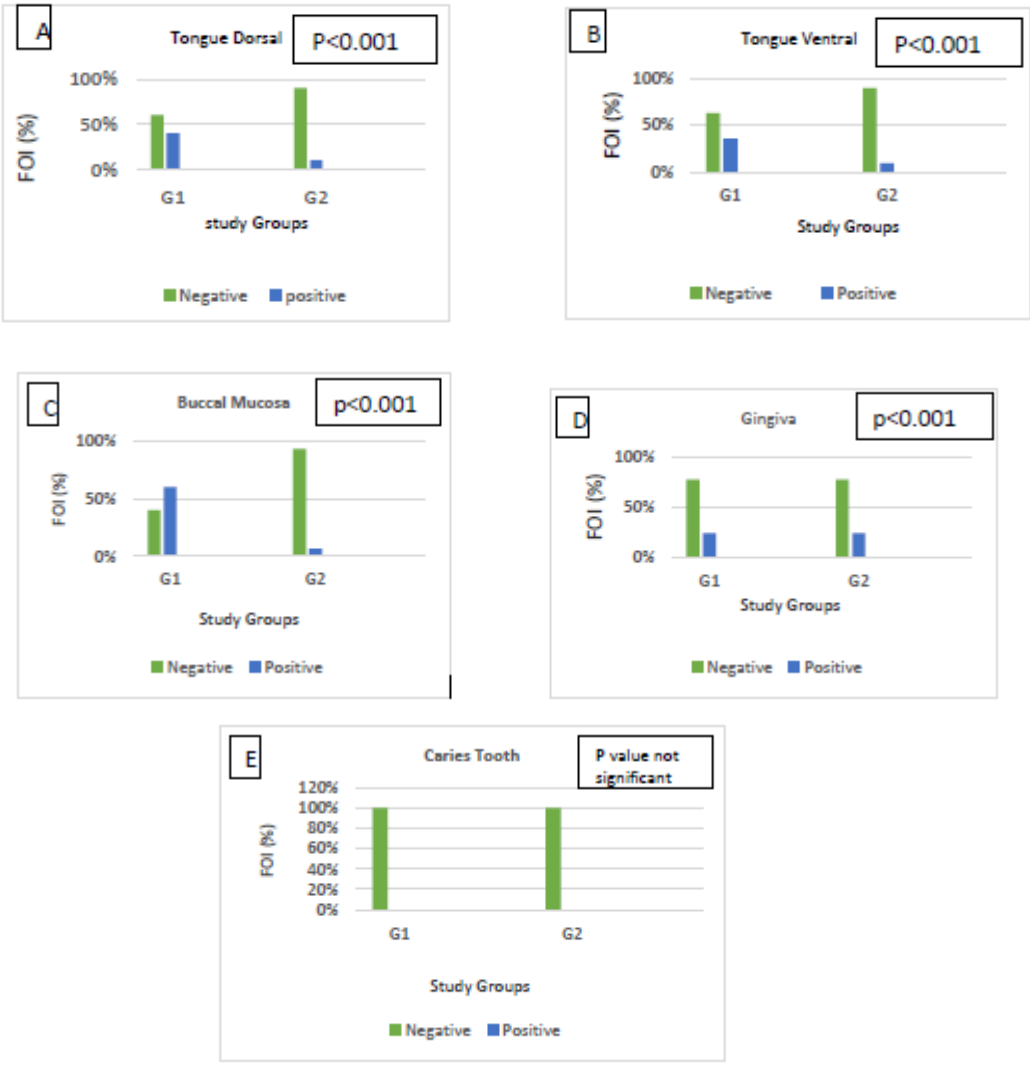
OR=21[3.71- 155.93]

d		Gingiva		
		Negative	Positive	
GROUP	I	7	23	X^2=17.07
	II	23	7	**p<0.0001**

OR= 10.8[2.84-43.82]

e		Carious Tooth	
		Positive	
GROUP	I	30	No comparison
	II	30	

Table.1 [a-e] show the number of samples that showed isolation of *Streptococcus mutans* in a group from each surface that is investigated & their level of significance between groups.



G1 – Group I, G2 – Group II plotted on the X – axis Graph Number: 1(a-e) show the number of samples that showed isolation of
Streptococcus mutans in a group from each surface that is investigated & their level of significance between groups

Table.2 shows the Mean & Standard Deviations of colony forming units of *Streptococcus mutans* isolated from the two subsets of patients in various surfaces.

	Number	*Dorsal Tongue*		*Ventral Tongue*		*Buccal Mucosa*		*Gingiva*		*Carious tooth*	
		Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
GROUP I	30	52.90	5.486	11.10	4.894	30.50	5.231	178.87	45.580	396.00	76.283
GROUP II	30	.83	.913	2.67	1.826	1.53	.973	22.67	8.438	37.70	15.038

Table.3 shows the significance level of the Colony forming units analysed for the study groups from the Dorsal surface of tongue.

Dorsal Surface Of Tongue – Colony Forming Units

Group	Group	Mean difference	Std. error	Significance
I	II	52.067*	.899	.000

Table.4 shows the significance level of the Colony forming units analysed for the study groups from the Ventral surface of tongue.

Ventral Surface of Tongue – Colony Forming Units

Group	Group	Mean difference	Std. error	Significance
I	II	8.433*	.811	.000

Table.5 shows the significance level of the Colony forming units analysed for the study groups from the Buccal Mucosa.

Buccal Mucosa – Colony Forming Units

Group	Group	Mean difference	Std. error	Significance
I	II	28.967*	.857	.000

Table.6 shows the significance level of the Colony forming units analysed for the study groups from the Gingiva

Gingiva – Colony Forming Units

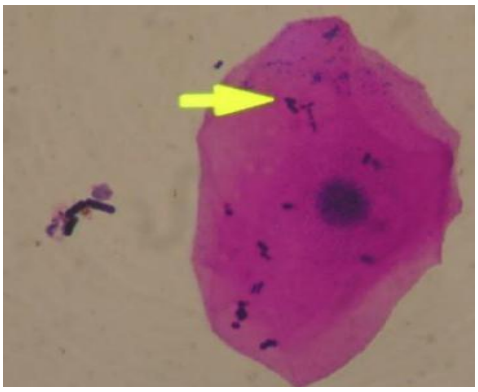
Group	Group	Mean difference	Std. error	Significance
I	II	156.200*	7.159	.000

Table.7 shows the significance level of the Colony forming units analysed for the study groups from the Carious tooth surface.

Carious Tooth Surface – Colony forming units

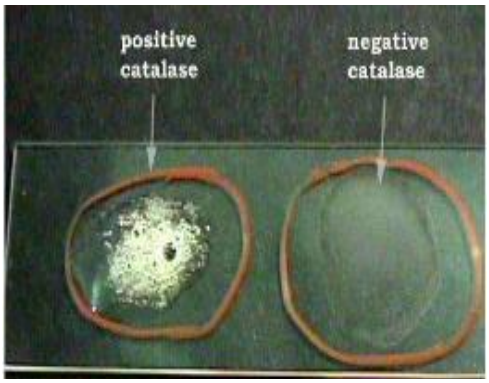
Group	Group	Mean difference	Std. error	Significance
I	III	358.300*	13.379	.000

Photograph.No.1 Shows Gram Stained Cytosmears Prepared from Patients of Three Study Groups Obtained from Buccal Mucosa



Photograph.No 2 Shows *Streptococcus mutans* Identified by Granular, Frosted, Glass Appearance in Mitis Salivarius Agar

Photograph.No 3 Show Catalase Positive and Negative Reactions



Photograph.No.4 Show *Streptococcus mutans* Colonies Exhibiting α – Haemolytic Pattern on Blood Agar

Therefore the probable reason to substantiate this is that in diabetes there is hypofunction of the salivary gland which leads to low salivary flow, reduction in pH of saliva and alteration in its composition (Piattelli *et al.,* 1989) or may be the reduction in the circulating insulin level in diabetes which leads to compensatory hyperplasia of the salivary glands thereby altering the salivary flow (Terry D Rees, 1994) this reason is also supported with the findings in literature (Kuo *et al., 2008*) stating that poorly controlled diseases has been associated with lower stimulated parotid flow.

Other reason could be also that changes in the hormonal, microvascular and neuronal in poorly controlled diabetes may lead to salivary gland hypofunction that alters the micro environment favourable for the growth of the organism. (Murrah, 1985; Gamboa *et al.,* 2008; Scully *et al.,* 2008; Sakeenabi *et al.,* 2011).

Whereas in Non diabetic [group II] the mean and standard deviation was almost less [Table Number: 2] when compared to Type II [Group I] the probable reason could be that the factor that normal salivary flow makes the environment self-cleansing or the immunoglobulin's present in saliva and gingival crevicular fluid that neutralizes the bacterial toxins by inhibiting the bacterial adhesions (Salonel *et al.,* 1990).

Considering the fact that fundamentals of Diabetes Mellitus and Dental caries remained common amongst our both diabetic groups, a higher yield in Type II Diabetes Mellitus would not be possibly substantiated by many reason that we know of. This made us to probe into the pathophysiology of Diabetes mellitus in itself so as to retrieve an explanation. Type II diabetes mellitus being a chronic low grade inflammatory metabolic disorder (Dandona *et al.,* 2004) are resultant of altered gut microbiota, leading to endoplasmic reticulum stress (Maximilian Zeyda Thomas, 2009) and altered metabolism of glucose in the target organs (Maximilian Zeyda Thomas, 2009) and in the metabolism of short chain fatty acids in the gut. (Davie, 2003; Gao *et al.,* 2009) This process leads to impairment and the release of mediators and endotoxins. (Wellen and Hotamisligil, 2005) In our study we considered that the altered microbiota could be a phenomenon that affects the whole of alimentary canal including oral cavity leading to yields which are unexpectedly high and different in Type II Diabetes Mellitus. Such a change could also be a reflection of endotoxins and mediators possibly due to their entry into systemic circulation.

The Frequency Of Isolation & Colony Forming Units of *Streptococcus mutans* was highest in carious tooth in type II diabetics [Table Number (1- e) and Table 7-(a& b)] respectively, followed by Gingiva **i**n type II

diabetes [Table Number 1 (d) and Table 6(a, b)] respectively. In carious teeth it is evident that the fact is the *Streptococcus mutans* will be definitely present because the food impaction or debilitated oral health will promote the resident flora to create an acidic environment, enhances fermentation, reduces the local pH rapid production of lactic acid from the dietary sugars aids in adhesion on the tooth surface causing dental caries. But as the samples in our study are related to diabetic we need to substantiate the role of diabetes and streptococcus and not the streptococcus and dental caries. Therefore it is the factor is that increased glucose level in saliva and gingival crevicular fluids, alters the plaque micro flora, and reduced salivary flow associated with the poor metabolic control could be the cause. (Terry, 1994; Kuo *et al.,* 2008; Ryan *et al.*, 1999; Tagelsir *et al.,* 2011) One more reason could be that the tooth surface is a non-shedding hard surface which selectively adsorbs various acidic glycoproteins such as mucins from the saliva which forms the acquired enamel pellicle which contains a high number of sulphate and carboxyl groups which directly increases the net negative charge of the tooth surface and its virulence factors that includes adhesins, glucan producing and binding exoenzymes proteases and cytokines stimulating molecules that helps in attachment (Marcotte and Lavoie, 1998; Zaura *et al.,* 2009) In diabetic the persistent pH drop after exposure to fermentable dietary carbohydrates can be due to the metabolic activity of increased numbers of bacteria on the tooth surfaces which leads to demineralization of the tooth which progress to cavitation of tooth. One more factor is the saliva which is the principal defence factor of the mouth is important for maintaining good oral health which is compromised by the prevalence of hypo salivation and xerostomia in type II diabetes that are predisposed to dental caries and growth of *streptococcus mutans* (Terry, 1994; Kuo *et al.,* 2008; Murrah, 1985; Rao *et al.,* 2010) In contradictory there is a study stating that there is no effect of diabetes on the prevalence of caries where the caries protective effect of saliva was partially lost in the patient with Non –Insulin dependent diabetes mellitus. Apart from this one more study states that in early gingivitis an ecological niches may be created for the growth of *Streptococcus mutans* along with other higher counts of cariogenic species and also may be due to the lower oxygen tension within the periodontal pockets that favours the growth of micro aerophilic species such as *Streptococcus mutans* (Canepari, 1919).

On the Ventral surface of Tongue the Frequency of isolation and Colony forming units of *Streptococcus mutans* was more in Type II diabetic patients. [Group I] compared to Non diabetic population[Group II]. [Table Number: 3(a, b)] the probable reason could be that it is a self - cleansing area with good salivary flow in the floor of the mouth compared to other areas and one more factor is that it is a shedding surface which prevents the colonization of the bacteria. This observation may hold true as the Colony forming units for *Streptococcal mutans* in the floor of the mouth and Ventral surface of the Tongue in normal individuals are also less.

The Frequency of isolation & Colony forming units of *Streptococcus mutans* was comparatively least from Buccal mucosa & Dorsal surface of Tongue respectively in Non diabetic [Group II]. (Table1c & Table 1a) & (Table 5 (a, b) & (Table 3 a, b) for the reason due to continuous programmed desquamation process, self-cleansing properties and the immunoglobulins which are the components of saliva that are responsible for anti- bacterial activity which prevents the colonization of the microorganisms.

In conclusion, an overview shows significant difference in isolation and yield of *Streptococcus mutans* between the Type II Diabetes Mellitus and Non Diabetes Mellitus group of subjects from all the surfaces studied except for Ventral surface of Tongue. The isolation was as expected, the same from

Caries tooth, in both the study groups but yield being higher in Diabetes group. Gingiva was the next frequent surface yielding more colonies. The study thus importantly highlights the yield of *Streptococcus mutans* for reasons is higher in Type II Diabetes Mellitus although Dental caries is common in both groups. We have studied only a species of Streptococci and a study on wider range of selective groups of organisms will open up our view point in this area.

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