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Dentistry Section

Salivary Factors, Candidal Colonization and Strain Diversity in Type II Diabetics

LAVANYA KALAPALA, VANDANA RAGHUNATH, AJAY BERNARD REGINALD

ABSTRACT

Introduction: Diabetes mellitus is one of the most common metabolic disorders of 21st century, occurring due to defect in insulin secretion or action resulting in chronic hyperglycemia. It is associated with altered salivary composition and function due to potential of insulin to alter metabolism in acinar cells. Diabetes, being an immunodeficient state is susceptible infections of which *Candida* is very frequent.

Aim: To correlate salivary glucose, total protein and amylase levels, oral candidal carriage and strain diversity in type II diabetics.

Materials and Methods: Salivary parameters, candidal carriage and strain diversity were assessed in 30 controlled and 30 uncontrolled type II diabetic individuals and 30 healthy controls. Glucose, total protein and amylase levels of unstimulated whole saliva were estimated by Glucose oxidase, dye binding and direct substrate kinase methods respectively; Candidal carriage and species identification by oral rinse and CHROMagar culture methods.

Results: Uncontrolled diabetics showed significant increase of all salivary parameters and candidal carriage ($p < 0.001$) compared to other groups. Non *Candida albicans* species were isolated in higher frequency (78.88%) in uncontrolled diabetics, with *C. tropicalis* (47/90) dominating. Significant correlation existed between all the salivary parameters and candidal carriage from healthy to diabetic states.

Conclusion: The consistent and significant increase of salivary parameters, candidal carriage and Non albicans *Candida* species in uncontrolled diabetics reflected the severity of the diabetic status implicating their diagnostic and prognostic merits. The correlation observed substantiated the role of salivary parameters in increasing candidal adherence.

Keywords: *Candida* species, Oral candidal carriage, Salivary glucose, Salivary total protein, Salivary amylase

INTRODUCTION

Diabetes mellitus (DM) is a common metabolic disorder as a result of absolute or relative lack of insulin. It is characterized by constant hyperglycemia with associated alterations in carbohydrate, protein and lipid metabolism [1]. According to International Diabetes Federation (IDF), type II diabetes is the most common type, currently afflicting 366 million population worldwide [2] and 50.8 million in India [3].

Being a systemic disorder, it is associated with altered salivary composition and function, disrupting the homeostasis of the oral cavity. Increased basement membrane permeability of salivary glands and vasculature, results in increased percolation of components from blood into saliva [4]. Of all the components salivary glucose, total protein and amylase levels appear to be more altered significantly in diabetes [5].

Oral candidal carriage rate is reported to be increased among the diabetic individuals predisposing them to candidal infections. Non-*Candida albicans Candida* species (NCAC's), despite their low virulence, are also commonly isolated in higher frequency in immunodeficient conditions and DM is one of them [6]. Additionally elevated salivary glucose, total protein and amylase are known to favor the growth and adhesion of these yeasts by promoting co - aggregation and co - adhesion with bacteria [7].

An extensive search using "Google Scholar" and "Pub Med" showed controversial observations pertaining to salivary amylase and total protein levels in type II diabetics, with only a few studies correlating salivary glucose, total protein levels and oral candidal carriage rate but hardly any in the Indian context. Further search failed to retrieve any reports of *Candida albicans* (CA) and non-*Candida albicans Candida* species (NCAC) studied in the context of the diabetic state, as reflected by the HbA1c levels.

The aim of our study was to assess and correlate the salivary levels of glucose, total protein and amylase and oral candidal carriage with strain diversity in type II diabetics; the diabetic state assessed by HbA1c levels and categorized further.

MATERIALS AND METHODS

Study Population: Individuals pertaining to this case control study were recruited from the outpatient departments of Narayana Dental and Medical Hospitals, Nellore, Andhra

Pradesh, India during the period of one year i.e., 2013 - 2014. Sample was selected from the population by stratified random sampling method. Informed consent was obtained from each individual of the study and the protocol was approved by the Institutional Ethical Committee of Narayana Dental College and Hospital, Nellore, India.

A total of 60 age and sex matched diabetic individuals of age ranging between 35 – 60 years were included in the study, who were further grouped, based on HbA1c percentage (Precipitation Method) into 2 groups of 30 each: controlled diabetics (HbA1c; 6 – 6.5%); uncontrolled diabetics (HbA1c: > 6.5%). Individuals for the control group constituted 30 healthy non diabetic individuals, confirmed with HbA1c percentage of < 6%.

The following selection criteria were included in the study: (a) Diabetic (Non-insulin dependent, Type II) individuals, diagnosed by the medical faculty as per the criteria established by the Committee on Diagnosis and Classification of Diabetes Mellitus (1998), with 2 – 5 years duration of diabetes; and (b) All individuals belonging to the same race, ethnicity, socio-economic status and living standards.

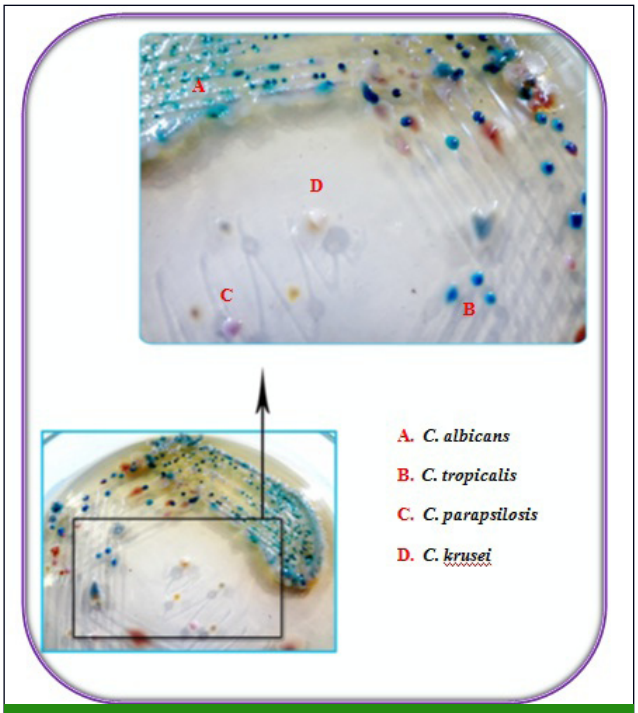
The patients with the following criteria were excluded from the study: (a) Diabetic individuals with complications (retinopathy, peripheral neuropathy and nephropathy etc) / oral lesions other than periodontitis and dental caries / other systemic complications / on medications other than those prescribed for diabetes (especially on NSAIDs, antifungal, antibiotics and Immuno suppressants); (b) Individuals with either partial or full removable or fixed prostheses; and (c) Individuals habituated to smoking / smokeless forms of tobacco.

Saliva Collection: Unstimulated whole saliva samples were collected 2 hours after breakfast, from the study individuals sitting in upright position. Samples were collected by spitting, into a wide mouthed sterile graduated plastic container which was then transferred immediately to sterile cuvettes and transported to the laboratory, within 2 hours of sampling.

Estimation Of Salivary Glucose, Total Protein And Amylase Levels: Saliva samples were centrifuged at 3000 rpm for 20 minutes and the clear supernatants obtained were processed further for the salivary parameters. Salivary glucose, total protein and amylase levels were estimated by GOD – POD, Dye binding and direct substrate kinase methods respectively (Human GM BH Henry Kits, Germany).

1000µl of the corresponding reagent solution was pipetted out into each of the 3 test tubes labeled as Blank, Standard and Test. Then 10µl of the corresponding standard solution was added to the test tube marked as 'Standard', followed by adding 10µl of the test sample to the test tube labelled as 'Test'. All the test tubes were vortexed manually and were kept in the incubator at 37ºC for 10 minutes. The solutions from all the test tubes were analyzed by a semi automatic analyzer (Humalyzer 3000, Germany) for salivary glucose, total protein and amylase levels and the reading were recorded.

Assessment of *Candida*: Saliva samples for candidal culture were obtained using oral rinse technique with phosphate buffer solution (pH - 7.2; 0.1mol/L). The rinse was collected in sterile plastic containers, then transferred to centrifuge tubes and concentrated by centrifuging at 1700 rpm for 10 minutes. The supernatants were discarded. An inoculation loop (4mm) full of the sedimented sample was taken and spread on CHROMagar *Candida* culture plates (Hichrome CHROMagar *Candida*). The plates were then labelled and incubated at 37ºC. Growth of *candida* species was observed after 24 - 48 hours of incubation. Colonies were quantified based on calibrated loop method of Myers and Koshi (1986). They were scored as 0 - absent (no colonies), 1 - very sparse (< 101 CFU/ml), 2 - sparse (101 – 102 CFU/ml), 3 - moderate (102 – 103) and 4 - rich (> 103). Species were recognized based on color index chart given in the manufacturer's manual and classified as *C. albicans* – green, *C. dubliniensis* – dark green, *C. tropicalis* – dark blue green with purple halo, *C. parapsilosis* – pale pink, *C. glabrata* – dark pink and *C. krusei* – pale pink center with rough spreading colonies [Table/Fig-1].



[Table/Fig-1]: CHROMagar *Candida* medium showing various *Candida* species
(*C. albicans, C. tropicalis, C. parapsilosis and C. krusei*)

STATISTICAL ANALYSIS

All the study variables were statistically analyzed using SPSS 20.00 software version. All the values were expressed as mean ± standard deviation (SD). Diabetic groups were compared with healthy controls using Student's t – Test. Intergroup comparisons were done by Post Hoc test (Tukey HSD). Relationship between the variables, such as salivary parameters and candidal carriage rate, was evaluated by

Spearman rank order correlation coefficient (rho). p-values of ≤ 0.05 and ≤ 0.01 were considered to be significant and highly significant respectively.

RESULTS

The age and sex demographics are shown in [Table/Fig-2]. The mean HbA1c percentage for uncontrolled diabetics (UCD) was 8.29 %, 6.24% for controlled diabetics (CD) and 5.32% in healthy controls (HC), which suggested that UCD had relatively poor metabolic control of the disease.

Salivary Parameters: We observed higher mean values of salivary glucose, total protein and amylase levels in UCD (8.26 mg/ml, 6 mg/ml and 4416.6 µg/L respectively), followed by CD and HC, which yielded a high statistical significance (p<0.001) on intergroup comparison, excepting for salivary amylase on comparing between CD and HC [Table/Fig-3,4]. Diabetics (CD and UCD combined) showed significantly higher (p<0.001) mean salivary glucose, total protein and amylase levels (5.747 mg/ml, 5.015 mg/ml and 3378.87 µg/L respectively) compared to HC [Table / Fig-5].

Oral Yeast Counts: We observed higher mean score of candidal CFU's in UCD (3.87), followed by CD and HC. A high statistical significance (p<0.001) on intergroup comparison was observed excepting between CD and HC groups [Table/Fig-3, 4]. Candidal carriage rate was higher in diabetics (3.35) compared to HC, which was highly statistically significant (p<0.001) [Table/Fig -5].

Isolation Of Oral *Candida* Species: *C. albicans* was isolated from all the individuals (100%) of the study population.

Study groups		Age range (years)	Number of cases	Gender distribution	HbA1c (%) levels
Diabetic Group	Controlled Diabetics (CD)	40 – 60	30	M -15 F -15	6 – 6.5
	Uncontrolled Diabetics (UCD)	38 – 60	30	M -15 F -15	> 6.5
Healthy Controls (HC)		36 - 60	30	M -15 F -15	< 6

[Table/Fig-2]: Age and sex demographics

Study Groups	T glucose Mean (mg/ml) ± SD	Total protein mean (mg/ml) ± SD	Amylase Mean (µg/l) ± SD	Candidal carriage rate (±CFU/ml)
CD	2.68± 0.646	4.02± 0.563	2343.14 ± 459. 25	2.87 ± 0.776
UCD	8.26 ± 3.16	6 ± 0.562	4416.6 ± 220.49	3.87 ± 0.346
HC	0.83 ± 0.317	1.5 ± 0.74	1730.3 ± 415.78	2.33 ± 0.922

[Table/Fig-3]: Mean values of salivary parameters and candidal carriage rate
†SD: Standard Deviation; ± CFU/ml: Colony forming units/ml

Study parameters	Intergroup Comparision	p-value
Salivary Glucose	CD & UCD	*** 0.001
	UCD & HC	***0.001
	CD &HC	***0.001
Salivary Total Protein	CD & UCD	***0.001
	UCD & HC	***0.001
	CD & HC	***0.001
Salivary Amylase	CD & UCD	***0.001
	UCD & HC	***0.001
	CD & HC	0.17
Oral Candidal Carriage Rate	CD & UCD	***0.001
	UCD & HC	***0.001
	CD & HC	0.15

[Table/Fig-4]: Intergroup comparisons of mean levels of salivary parameters and candidal carriage rate
***p value < 0.001 - high statistical significance

Study Parameters	Diabetics (Mean ± SD)	Healthy Controls (Mean ± SD)	p - value
Salivary Glucose	5.747 ± 3.61	0.83 ± 0.317	***0.001
Salivary Total Protein	5.015 ± 1.144	1.5 ± 0.742	***0.001
Salivary Amylase	3378.87 ± 1893.21	1730. 3 ± 415.78	***0.001
Oral Candidal Carriage Rate	3.35 ± 0.77	2.33 ± 0.922	***0.001

[Table/Fig-5]: Mean values of salivary parameters and candidal carriage rate in diabetic and healthy control groups
†SD: Standard Deviation; *** p ≤ 0.001 – highly significant

However non-*Candida albicans Candida* species (NCAC's) were isolated in higher frequency in UCD (100%), followed by CD (90%) and HC (46.6%), but was not statistically significant only between UCD and HC. Statistical significance was observed between diabetics as a whole and HC [Table/Fig -6, 7]. *C. tropicalis* (52.2%) was the predominant species isolated in all the groups, followed by *C. glabrata* (36.7%), *C. parapsilosis* (31.1%) and *C. dubliniensis* (23.3%). *C. krusei* (5.6%) was noted only in diabetics [Table /Fig-7].

Correlation Between Individual Salivary Parameters And Candidal **Carriage Rate:** A significant positive (0.001) correlation was observed on correlating candidal carriage rate with all the salivary parameters (glucose, total protein and amylase) (rho: 0.530, 0.577 and 0.374 respectively) with the worsening of diabetes state (HC CD UCD) [Table/Fig- 8-10].

DISCUSSION

The present study was carried out to investigate the effect of type 2 diabetes on few relevant salivary parameters and oral

Study Groups	*C. albicans* n (% = n/30)	NCAC species n (% = n/30)	NCAC species in diabetics n (% = n/30)
CD (30)	30 (100%)	27 (90%)	57 (95%)
UCD (30)	30 (100%)	30 (100%)	
HC (30)	30 (100%)	14 (46.6%)	14 (46.6%)

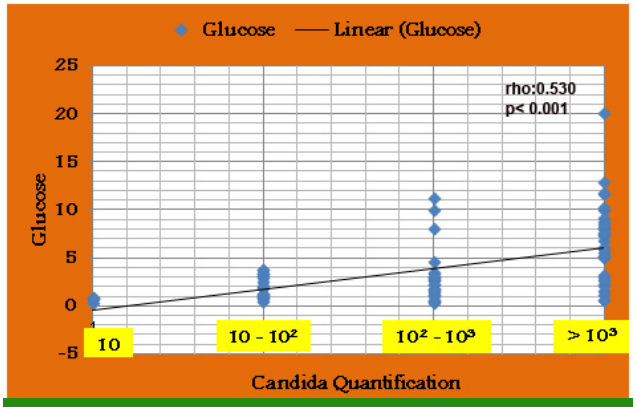
[Table/Fig-6]: Percentage distribution of *Candida albicans* and Non *Candida albicans Candida* Species (NCAC's) amongst study groups

candidal carriage rate, both in the context of controlled and uncontrolled diabetic states, as assessed by HbA1c levels. Very few studies in this regard have been done with conflicting results.

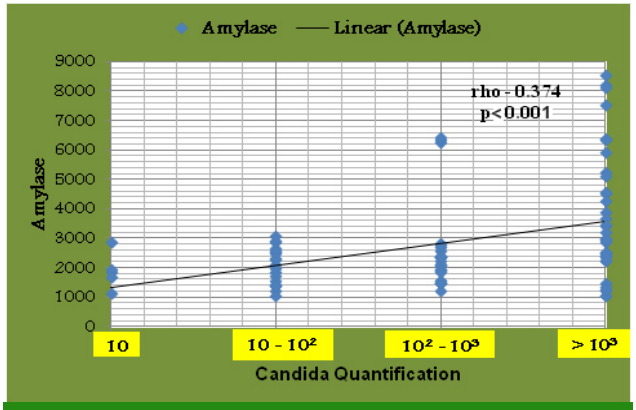
Our observations high mean salivary glucose levels in Uncountrolled diabetes (UCD) compared to Controued diabetes (CD) and Healthy Controls (HC), were similar to very few observations of significantly [5, 8-10]. However, Shukria M. et al.,[9] and Arati S Panchbhai et al., [5] did not observe statistically significant differences between UCD and CD. The diabetic status in these studies were categorized based on HbA1c [9] and random blood glucose levels [5]. Diabetics

Study Groups	C.albicans n (%= n/30)	Non *Candida Albicans Candida* Species (NCAC's)				
		C. glabrata n (%= n/30)	*C. tropicalis* n (%= n/30)	*C. parapsilosis* n (%= n/30)	*C. dubliniensis* n (%= n/30)	*C. krusei* n (%= n/30)
CD (30)	30 (100%)	13 (43.3%)	17(56.7%)	10 (33.3%)	7 (23.3%)	2 (6.7%)
UCD (30)	30 (100%)	17 (56.7%)	23 (76.7%)	15 (50%)	12 (40%)	3 (10%)
HC (30)	30 (100%)	3 (10%)	7 (23.3%)	3 (10%)	2 (6.7%)	0 (0%)
Total (90)	90 (100%)	33 (36.7%)	47 (52.2%)	28 (31.1%)	21 (23.3%)	5 (5.6%)

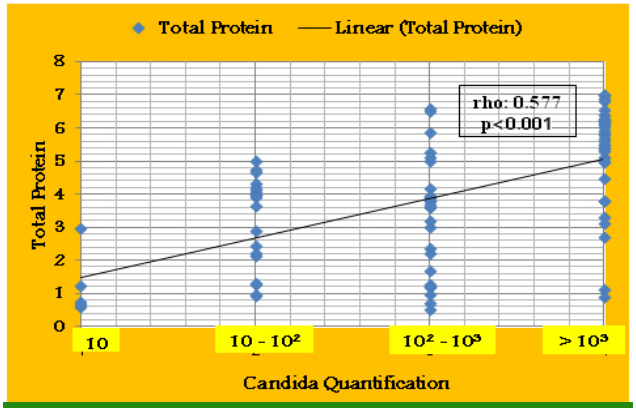
[Table/Fig-7]: Percentage distribution of individual candidal species amongst study groups



[Table/Fig-8]: Correlation between salivary glucose levels and candidal carriage



[Table/Fig-10]: Correlation between salivary amylase levels and candidal carriage



[Table/Fig-9]: Correlation between salivary total protein levels and candidal carriage

showed significantly higher mean salivary glucose levels than healthy controls, similar to many previous findings [2, 11-16], except that of Anupama Hegde et al., [17], Bakianian Vaziri et al ., [18], Peiro Marchetti et al., [19] and Andelski Biljana et al., [20]. Variations in mean values of salivary glucose observed in the above studies could be due to difficulty in standardizing the procedures of time and type of saliva collection, blood and salivary glucose estimation methods, the difference in duration of diabetes, glycemic control status, life style and socioeconomic status of the patients [9, 17, 21]. High mean salivary glucose levels observed in HC could be due to the carbohydrate rich diet pattern of Indian population [10].

Statistically high mean salivary total protein levels in UCD, followed by CD and HC (i.e. UCD > CD > HC) observed in our study, stood in contrast to the only two studies of Shukria M. et al., [9] (CD > UCD > HC) and Arati. S. Panchbhai et

al., [5] (HC > UCD > CD). However significantly high levels of total protein observed in diabetics as a whole than in controls, were similar to the very few studies by Ezel Yavuzyilmaz et al., [22], Praveen Kumar Shetty et al., [23] and Dodds et al., [24]. Though many other studies observed high salivary total protein levels in diabetics [13,14,16,25,26] the results were statistically insignificant. On the contrary Kumar et al., [27] and Streckfus et al., [28] noted lower total protein levels in diabetics than in non diabetics. Thus only few studies correlate with our observation in diabetics and none of the two studies categorizing the diabetic state. Variations in saliva sampling (either whole unstimulated/stimulated saliva), salivary flow rate, methods of determination of total proteins and diurnal variations could have perhaps played a role [19].

Mean salivary amylase levels were observed to be higher in UCD, followed by CD and HC, yielding a high statistical significance, excepting between CD and HC. This was in accordance to the only two studies of Sathya Priya et al., [29] and Shankariah et al., [30] but conflicted with that of Shukria M. Al. Zahawi et al., [9] observation (HC > UCD > CD). Diabetics as a whole showed significantly higher values compared to HC, which was similar to Suleyman Aydin et al., [13] and Dodds et al., [24] findings. Though Carmen Carda et al., [14] and Newrick et al., [31] recorded high salivary amylase levels, the difference was not apparent statistically. Contrastingly Ezel Yavuzyilmaz et al., [22] observed significantly lower levels in diabetics, which was attributed to other hormonal and metabolic changes, affecting the salivary composition.

Increase in levels of salivary glucose, total protein and amylase in diabetics follows basement membrane structural alterations of the parenchymatous component of salivary glands and stromal microvasculature of both salivary glands and gingiva, resulting in increased percolation of salivary and serum components [2]. Salivary samples collected in the present study represented complete mouth fluid, reflecting the components derived from both the salivary glands and the gingival crevicular fluid. Unstimulated salivary samples are preferred for evaluating salivary components, as it is known that stimulation affects both the quantity and quality i.e. pH and concentration of certain salivary constituents particularly total protein [32]. Xerostomia, being one of the complications of diabetics definitely concentrates the salivary components [1]. Very few studies [5,14] have assessed salivary flow rate in diabetics but have not attempted to correlate it with salivary glucose, total protein and amylase levels. It is always better to study the salivary parameters in context of flow rate which was one of the shortcomings of our study.

Mean candidal carriage rate was higher in UCD followed by CD and HC, similar to Radhika Sashikumar et al., [10] and Suarez et al., [33] observations. We noted a high statistical difference on intergroup comparison excepting between CD and HC, while Radhika Sashikumar et al., [10] observed between all the three groups and Suarez et al., [33]] failed to note between UCD and CD. Diabetics as an entirety showed significantly higher candidal carriage rate than HC, which was in accordance with the studies of Safia A et al., [34], Kumar et al., [35] and Willis et al., [36], but Darwazeh et al., [37], Lamey et al., [38] and Fischer et al., [39] didn't observe any statistical significance. Flavia Cristina Volpato et al., [40], on the contrary noted higher candidal carriage rate in HC. Differences in mean candidal carriage rate among various studies could be due to slight variations in the oral rinse sample method itself and oral hygiene status as reflected by the local factors like periodontitis, presence of dentures, smoking etc [24]. Our study was standardized to certain extent by excluding denture wearers and smokers. However the contribution of tenaciously adherent plaque to candidal carriage rate needs to be studied further. Concentrated oral rinse technique is considered as the best method compared to other methods and also dislodges the plaque to some extent [41]. Several studies using methods like imprint / whole saliva / impression / swab / smear cultures have shown a high candidal carriage rate in diabetics, but with varying statistical significance indicating that methodology also influences the candidal carriage rate [42,43].

Candida being an opportunistic pathogen, frequently colonizes multiple sites in the oral cavity and requires the commensal bacteria for adherence. Glycation induced alteration in T – cell functions, impaired intracellular killing and opsonization of granulocytes, all which help in extensive proliferation of oral bacteria which in turn participate in co - aggregation and co - adhesion of *candida*, thus increasing the candidal carriage rate in diabetics [24, 44]. Further in diabetics, the enzyme transglutaminase which gets elevated concomitantly with increased levels of advanced glycation end products (AGE's), enhances the binding of candidal hyphal proteins with the surface of epithelial cells [45]. Additionally reduced salivary flow rate and impaired flushing action of saliva further promotes candidal colonization in diabetics [24, 44].

C. albicans was isolated in all the individuals of the study irrespective of diabetes, which was in contrast to many previous studies, where higher percentage of isolation was noted only in diabetics. *C. albicans* is a known commensal of the oral cavity and oral hygiene status could have attributed, which was not considered in our study.

NCAC's with *C. tropicalis* being the predominant species was observed in higher frequency in UCD followed by CD and HC. Many studies have noted NCAC's, but none of them have studied them in the context of the diabetic state and variations among predominant NCAC's too have been observed. Contrary to our observation of high NCAC isolation in diabetics (UCD and CD combined) Manfredi et al., [46] and M. Bharazhi et al., [47] observed it in HC. Despite their lower adherence capability to buccal epithelial cells and secretion of lesser amounts of proteinases, NCAC's are considered as potential oral colonizers probably due to growing population with immunocompromised conditions like diabetes mellitus, who are more prone to colonization or infection by these atypical species [6].

One of the main objectives of our study was to correlate the salivary parameters with the candidal carriage rate in diabetics.

On correlating salivary glucose levels and candidal carriage rate, we observed a significant positive correlation similar to the observations of the only two studies of Darwazeh et al., [37] and Radhika Sashikumar et al., [10] in diabetics. High salivary glucose levels increase candidal adherence to buccal epithelial cells by forming reversible glycosylation end products with tissue proteins, which in turn leads to accumulation of glycosylation products, thus increasing the receptors for *Candida* [10]. Neutrophils produce less free oxygen radicals, particularly in presence of glucose thereby leading to defective *candida*cidal activity which thus helps in increased candidal colonization [48].

Similarly, significant positive correlation between salivary total protein levels and candidal carriage rate was observed. No studies in English literature (search in "Google Scholar" and "PubMed") have attempted such a correlation. Salivary total protein levels though get elevated in diabetics; their antimicrobial effectiveness gets impaired by glycation [24], allowing excess proliferation of bacteria which act as primary colonizers / receptors. These along with other salivary proteins like lysozyme, lactoferrin, salivary peroxidase, mucins and glycoproteins, enable increased candidal co - adhesion and co – aggregation. Further, candidal proteinases also bring down the antimicrobial effectiveness of salivary proteins through their proteolytic action [49]. Thus all the above factors act indirectly in influencing the candidal carriage except for transglutaminase protein.

Salivary amylase though not related to any specific anti *candida* cidal action, on combining with other antimicrobial proteins such as proline rich proteins, IgA and alpha amylase, function as receptors on precipitation to the oral tissues, promoting microbial and fungal adhesion [7]. Interestingly we did observe a significantly positive correlation between salivary amylase levels and candidal carriage rate.

CONCLUSION

The clinical significance of this study is that significant rise in the salivary glucose, total protein and amylase levels in uncontrolled diabetics, followed by controlled diabetics and healthy controls indicate that the levels of these salivary parameters increase parallely with glycemic status of the diabetic individuals. Further studies abiding by the standardization protocols could make these as useful adjuncts for diagnosing and monitoring the diabetic status. But the role of the salivary parameters, mainly total protein on candidal carriage rate as observed in our study needs to be further explored. Further studies with larger sample size are required to confirm the finding, which is the drawback of this study.

REFERENCES

[1] Negrato CA, Tarzia O: Buccal alterations in diabetes mellitus. *Diabetology & Metabolic Syndrome* 2010; 15:2:3.

[2] Abikshyeet P, Ramesh V ,Oza N: Glucose estimation in the salivary secretion of diabetes mellitus patients. Diabetes, Metabolic syndrome and Obesity: *Targets and Therapy* 2012; 5: 149 – 154.

[3] Yadav NK, Sathian B, Kalai RS: Assessment of Diabetes Mellitus in India and Nepal. *Biochemistry* 2012; 3(6):WMC003544

[4] Murrah VA, Crosson JT, Sauk JJ: Parotid gland basement membrane variation in diabetes mellitus. *Journal of Oral Pathology* 1985: 14: 236-246

[5] Panchbhai AS, Degwekar SS, Bhowte RR: Estimation of salivary glucose, salivary amylase, salivary total protein and salivary flow rate in diabetics in India. *Journal of Oral Sciences*, 2010;52(3):359-68.

[6] Meurman JH, Siikala E et al..Non *candida albicans candida* yeasts of the oral cavity. Communicating Current Research and Educational Topics and Trends in Applied Microbiology, A Mendez Vilas ed, Formatex, 2007

[7] Jenkinson HF and Douglas LJ: Text Book of Polymicrobial Infections; Chapter – 18: Interactions between *Candida* species and bacterial in mixed infections.

[8] Panchbhai AS: Correlation of Salivary Glucose Level with Blood Glucose Level in Diabetes Mellitus. *J Oral Maxillofac Res.* 2012; 3(3):e3

[9] Shukria M. Al-Zahawi, Hassan A. Mahmood Al-Barzenji, Zewar A. Al-Qassab: Effects of diabetes mellitus types II on salivary flow rate and some salivary parameters (total protein, glucose, and amylase) in Erbil city. *J Bagh Coll Dentistry* 2012; 24(Sp. Issue 2):123-27

[10] Sashikumar R and Kannan R: Salivary glucose levels and oral candidal carriage in type – II diabetics. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2010; 109; 706 – 11.

[11] Vasconcelos AC, Soares MS, Almeida PC, Soares TC. Comparative study of the concentration of salivary and blood glucose in type 2 diabetic patients. *Journal of Oral Science*. 2010;52(2):293-98.

[12] Sreedevi, Shashikanth MC and Shambulingappa P. Comparision of serum glucose and salivary glucose in diabetic patients. *Journal of Indian Academy of Oral Medicine and Radiology*.2008; 20(1).

[13] Aydin S: A comparison of ghrelin, glucose, alpha-amylase and protein levels in saliva from diabetics. *Journal of Biochemistry and Molecular Biology*.2007;40(1): 29-35.

[14] Carda C, Mosquera-Lloreda N, Salom L, Gomez de Ferraris ME, Peydró A. Structural and functional salivary disorders in type 2 diabetic patients. *Med Oral Patol Oral Cir Bucal* 2006; 11: E309-14.

[15] Thornstensson H, Falk H, Hugoson A and Olsson J: Some salivary factors in insulin dependent diabetics. *Acta Odontol Scand* 1989; 47(3): 175 – 83.

[16] Harrison R and Bowen WH : Flow rate and organic constituents of whole saliva in insulin dependent diabetic children and adolescents. *Pediatric Dentistry*. 1987; 9(4):287-91.

[17] Hegde A, Shenoy R, D'Mello P, Smitha A, Tintu A, Manjrekar P: Alternative markers of glycemic status in diabetes mellitus. *Biomedical Research.* 2010; 21 (3): 252-56.

[18] P. Bakianian Vaziri, M. Vahedi, H. Mortazavi et al: Evaluation of salivary glucose, IgA and flow rate in diabetic patients: A case control study. *Journal of Dentistry, Tehran University of Medical Sciences, Tehran, Iran*. 2010; 7(1):13-18.

[19] Marchetti P, Tognarelli M et al: Decreased salivary glucose secretory rate: usefulness for detection of diabetic patients with autonomic neuropathy. *Diabetes Research and Clinical Practice*, 1989;7: 181 –86.

[20] Radicevic Biljana, A., Silvija M et al. Changes in biochemical composition of saliva in diabetic patients. *Serbian Dental Journal*. 2006; 53.

[21] Ivanoski K, Naumovski, Koastadinova et al. Xerostomia and salivary levels of glucose and urea in patients with diabetes. contributions, Sec. *Biol. Med. Sci, MASA*. 2012;33(2):219-29.

[22] Yavuzyilmaz E, Yumak O, Akdoğanli T, Yamalik N, Ozer N, Ersoy F, Yeniay I. The alterations of whole saliva constituents in patients with diabetes mellitus. *Australian Dental Journal.* 1996 Jun;41(3):193-97.
[23] Shetty PK and Pattabiraman TN: Salivary proteolytic activities in periodontitis, gingivitis and diabetes mellitus. *Indian Journal of Clinical Biochemistry*. 1998; 13(1): 46 – 51.
[24] Dodds MW , Yeh CK, Johnson DA. Salivary alterations in type – 2 (Non Insulin Dependent) diabetes mellitus and hypertension. *Community Dent Oral Epidemiol.* 2000; 28(5): 373 –81.
[25] Lasisi TJ and Fasanmade AA: Comparative analysis of salivary glucose and electrolytes in diabetic individuals with periodontitis. Ann Ibd. *Pg. Med.* 2012;10(1):1 25-30.
[26] Javed F, Klingspor L, Sundin U, Altamash M, Klinge B, Engström PE. Periodontal conditions, oral *Candida albicans* and salivary proteins in type 2 diabetic subjects with emphasis on gender. *BMC Oral Health.* 2009;9:12.
[27] Kumar. J. Saliva – a marker for the diabetic: a comparative study of healthy and the diabetic individuals. *Indian J. Innovations Dev*. 2012;1(2).
[28] Streckfus C. F, Welsh S et al: Parotid function and composition of parotid saliva among elderly edentulous African – American diabetics. *J Oral Pathol Med.* 1994; 23; 277 – 79.
[29] Priya SS, Bharani GO, Nagalingam M, Jayanthi M, Kanagavalli U: Potential of salivary protein as a biomarker in prognosis of Diabetes mellitus. *Journal of Pharmacy Research.* 2011, 4(7), 2228-29.
[30] Shankaraiah P and Reddy YN. α – amylase expression in type – 2 diabetic patients. *J. Med Sci*. 2011;11(7):280 –84.
[31] Newrick PG, Bowman C et al. Parotid salivary secretion in diabetic autonomic neuropathy. *The Journal of Diabetic Complications* 5; 1: 35 – 37, 1991
[32] Kaufman E and Lamster IB: The diagnostic applications of saliva – a review. *Crit Rev Oral Biol Med.* 2002;13 (2);197–212.
[33] Suarez BL et al. *Candida* species and other yeasts in the oral cavities of type – 2 diabetic patients in Cali, Colombia. *Colombia Medica.* 2013;0;44(1):26-30.
[34] Safia A. Al – Attas, Soliman O. Amro: Candidal colonization, strain diversity and antifungal susceptibility among adult diabetic patients. *Ann Saudi Med.* 2010; 30(2): 101 –08.
[35] Kumar BV, Padshetty NS, Bai KY, Rao MS. Prevalence of *Candida* in the Oral Cavity of Diabetic Subjects. *JAPI*. 2005;53.
[36] Willis AM, Coulter WA et al. Isolation of *C. dubliniensis* from insulin using diabetes mellitus patients. *J Oral Pathol Med* 2000; 29: 86 – 90.
[37] Darwazeh AM, MacFarlane TW, McCuish A, Lamey PJ. Mixed salivary glucose levels and candidal carriage in patients with diabetes meliitus. *J Oral Pathol Med.* 1991; 20(6): 280-83.
[38] Lamey PJ, Darwaza A, Fisher BM, Samaranayake LP, Macfarlane TW, Frier BM.Secretor status, candidal carriage and candidal infection in patients with diabetes meilitus. *J. Oral Pathol.* 1988: 17(7): 354-57.
[39] Fisher BM, Lamey PJ, Samaranayake LP, MacFarlane TW, Frier BM: Carriage of *Candida* species in the oral cavity in diabetic patients: relationship to glycaemic control. *J Oral Pathol.* 1987: 16: 282-84.
[40] Volpato FC, Pires JR et al: Prevalence of Candia species during radiographic examination in diabetes mellitus patients. *Rev Odontol UNESP*. 2013; 42(1): 13 – 19.
[41] Samaranayake LP, MacFarlane et al. A comparision of oral rinse and imprint sampling techniques for the detection of yeast, colliform and *Staphylococcus aureus* carriage in the oral cavity. *J Oral Pathol.* 1986; 15: 386 –88.
[42] White PL1, Williams DW, Kuriyama T, Samad SA, Lewis MA, Barnes RA. Detection of *Candida* in concentrated Oral rinse culture by Real Time PCR. *Journal of Clinical Microbiology*. 2004;42(5):2101-07.
[43] How VJL and Parasakthi N: Urinary viable counts – an evaluation of three methods. *Malaysian J. path.* 1978; 1: 77 – 84.
[44] Khazal FA, Mahran A, Hasnawi HHA. Oral carriage rate of *Candida* species in diabetic patients. *Al Kindy Col Med J.* 2006;3(1): 9-12.
[45] Bradway SD: Do proline rich proteins modulate a transglutaminase catalyzed mechanism of candidal adhesion? critical Reviews in *Oral Biology and Medicine*.1993; 4(3/4); 293 – 99.
[46] Manfredi M, McCullough MJ et al: Invitro evaluation of virulence attributes of *Candida* species isolated from patients affected by diabetes mellitus. *Oral Microbiology Immunology.* 2006; 21: 183 – 89.
[47] Bharathi M, Rani AU, Sandhya C: A comparative study of carrier state of *candida* and its speciation in oral flora – among healthy individuals, persons with DM and HIV sero positive individuals. *Dermatol Online.* 2012; 3(2): 102-06.
[48] Soysa NS, Samaranayake LP and Ellepola ANB: Diabetes mellitus as a contributory factor in oral Candidosis. *Diabet. Med*. 2006;23, 455–59.
[49] Germaine GR, Tellefson LM, Johnson GL. Proteolytic activity of *candida albicans*: action on human salivary proteins. *Infection and Immunity*.1978;22(3): 861 -66.

AUTHOR(S):

1. Dr. Lavanya Kalapala
2. Dr. Vandana Raghunath
3. Dr. Ajay Bernard Reginald

PARTICULARS OF CONTRIBUTORS:

1. Senior Lecturer, Department of Oral & Maxillofacial Pathology, Drs Sudha and Nageswara Rao Siddhartha Institute of Dental Sciences, Gannavaram, India.
2. Professor and Head, Department of Oral and Maxillofacial Pathology, Narayana Dental College and Hospital, Nellore, India.
3. Professor, Department of Oral and Maxillofacial Pathology, Narayana Dental College and Hospital, Nellore, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Lavanya Kalapala,
D. No: 14 – 79, Hanuman Street, Tulasi Nagar, Ashok Nagar, Vijayawada, Andhra Pradesh-520 007, India.
E-mail: lavanya.kalapala39@gmail.com

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