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Caries Inhibition of Pit and Fissure

Real Obligation of Endoscopic Appraisal

Highlights

Influence of Metallurgy and File Design

Evaluation of Possible Probiotic Strain

Discovering Thoughts, Inventing Future

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Influence of Metallurgy and File Design in Micro Crack Formation in Root Canals - An in Vitro Study

By Dr. Jyothilekshmi B, Dr. Rajesh Pillai, Dr. N O Varghese, Dr. Afzal Abdul Salim, Dr. Sheila George, Dr. Nikhil Murali, Dr. Geetha Ramachandran & Dr. Praveena S V

Abstract- Aim and Objectives: This study aims to compare the incidence of dentinal microcracks produced by Max Wire Technology (XP Endo Shaper-single files), Adaptive Pitch (Hero shaper – multiple files) and Controlled Memory (Hyflex – multiple files) files during root canal procedures in single- rooted teeth using a stereomicroscope.

Materials and Methods: Sixty four single-rooted mandibular premolars with similar sizes and completed apices were selected. All specimens were decoronated using a diamond- coated bur with water cooling, leaving roots approximately 15 millimeter in length. Silicone impression material was used for covering the external surface of roots to simulate periodontal ligament space, and specimens were then embedded in the alginate impression material.

Keywords: cracks, controlled memory, dentinal damage, xp endo shaper, hyflex cm, hero shaper, max wire technology, adaptive pitch, controlled memory, root canal instrumentation, root fracture, rotary nickel-titanium instruments.

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Influence of Metallurgy and File Design in Micro Crack Formation in Root Canals- An in Vitro Study

Dr. Jyothilekshmi B [α], Dr. Rajesh Pillai [σ], Dr. N O Varghese [ρ], Dr. Afzal Abdul Salim [ω], Dr. Sheila George [¥], Dr. Nikhil Murali [§], Dr. Geetha Ramachandran [χ] & Dr. Praveena S V [ν]

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Interpretation and Conclusion: All rotary systems used in this in vitro study created dentinal cracks in root canals at all three levels, whereas XP ENDO SHAPER (max wire technology) presented with minimal dentinal microcracks but no significant difference was noticed.

Keywords: cracks, controlled memory, dentinal damage, xp endo shaper, hyflex cm, hero shaper, max wire technology, adaptive pitch, controlled memory, root canal instrumentation, root fracture, rotary nickel-titanium instruments.

I. Introduction

The irrevocable aim of endodontics is to create a three-dimensional flawless seal of the root canal system. [(1)] During endodontic treatment procedures, roots are susceptible to develop dentinal damage, quantity of which are influenced by numerous factors like physical properties of teeth, [(2)] preparation technique or various endodontic instruments that used, etc. The American Association of Endodontists classifies the longitudinal tooth fractures as five different types: [(3)] Craze lines, Cuspal fractures, Cracked tooth, Split tooth,Vertical root fracture.

Shemesh [(4)] et al. defined dentinal defects as all lines that appeared to disrupt the integrity of the dentin on the root end surface that extended either from the external root surface onto the resected dentin surface or from within the root canal lumen onto the resected root surface.

Despite the technological advancements, microcrack formation and vertical root fracture remain as significant problems during root canal shaping and cleaning procedures using Ni-Ti instruments [(5)]

Rotary systems are classified into single and multi-file systems. Preparation of the entire root canal using one single Ni-Ti instrument has many advantages, such as being cost-effective, decreasing cross-contamination, and reducing instrument fatigue. But it might be speculated that more stress will be generated during instrumentation when using only one instrument, thereby increasing the frequency of microcracks [(6,7)]

Hero Shaper [(8)] (HS; Micro-Mega, Besancon, France) is a multiple file system with a triple helix cutting edge and a safe ended tip with variation in the helical angle from the tip to the shank. Controlled memory (CM) wire (Coltene/ Whaledent, Altst€atten, Switzerland) made with thermally treated NiTi alloy has been introduced a few years back. Because of the austenite/martensite transformation as a result of heat treatment, CM wire has a stable martensitic microstructure at body temperature [(9)]

A recently introduced single file system known as the XP-endo Shaper[(10)] (XP) (FKG Dentaire, La Chaux-de-Fonds, Switzerland) uses a rotary Ni-Ti snake-shaped instrument. The Max Wire® and Booster Tip technologies combine to make the XP-endo Shaper a "One File Shaper." It has an initial taper of .01 in its M phase when it is cooled. Upon exposure to body temperature (35^{0}C), the taper changes to .04 according to the molecular memory of the A phase. XP achieves a final minimum canal preparation of 30/.04 when using this instrument alone. It applies minimal stress to the dentin walls, thereby minimizing the risk of microcracks

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in the dentin. XP can adapt to canal irregularities and has excellent resistance to cyclic fatigue

Till now, no study has evaluated the incidence of dentinal microcracks that result from the use of the XP system against Heroshaper and Hyflex CM. Hence, the purpose of the present study was to inspect the effects of using Hyflex CM, Hero Shaper, and XP files on the incidence of dentinal defect formation using a stereomicroscope.

II. Materials and Methods

Sixty-four single-rooted, human noncarious mandibular premolar teeth with similar anatomy and closed apices were selected. Using an ultrasonic scaler, soft tissues and calculus were removed from the root surfaces. Buccolingual and mesiodistal radiographs were taken from the specimens to verify the presence of single canal in each root. The teeth were temporarily stored in 4 °C distilled water till further use. All the roots were inspected with transmitted light and stereomicroscope under 8X magnification to detect any pre-existing craze lines or cracks. Teeth with anomalies were excluded from the study and replaced by intact teeth. Covered the external surface of roots with silicone impression material to simulate periodontal ligament space, and specimens were then embedded in the alginate impression material. Canal patency was established with a size no-: 15 K-File (Dentsply Maillefer, Ballaigues, Switzerland). The working length of the canals was determined by inserting a size 15 K-type file into the root canal terminus and subtracting 1 mm from this measurement.

III. Root Canal Preparation

Tooth were randomly divided into 3 experimental groups (Groups 1-3) and one control group with 16 teeth in each group. All teeth were measured and the crowns were sectioned with a high-speed bur under copious water spray in order to obtain equal lengths of the roots.

- Group I: No preparation

Sixteen teeth were left unprepared and served as controls.

- Group II: Teeth prepared with a single file system (XP ENDO SHAPER)((FKG Dentaire SA Switzerland)

In XP groups, root canals were enlarged with a K-file until #25 by manufacturers' recommendations. The XP file was first placed in 35^{0}C water and then placed in the root canal in order to enable phase transformation. Preparation time was one minute at a speed of 800 rotations per minute and 1- Newton cm torque according to the manufacturer's recommendations.

- Group III: Teeth prepared with multiple rotary file system (Hyflex CM rotary file)

A gentle in-and-out motion with a rotational speed of 500 rotations per minute, and 2.5 Newton cm torque was used to operate the HyFlex files. The sequence used was 20/0.04 (till two-thirds of the working length), 25/0.04, 30/0.04 (till full working length).

- Group IV: Teeth prepared with multiple rotary file systems (Heroshaper rotary file)

In the HeroShaper group, HeroShaper NiTi files were used in a crown-down sequence to file #30 at 450 rotations per minute at a torque of 1.2Newton cm. The order used was 20/04, 25/04, 30/04.

In all the groups, between each instrument, irrigants used were 3% sodium hypochlorite and 0.9% w/v Sodium Chloride. A total volume of 12 mL of sodium hypochlorite was required. EDTA in gel form was used as a lubricant.

a) Sectioning and Microscopic Examination

Sectioning of all the roots was performed perpendicular to the long axis at distance of 9, 6 and 3 mm from the apex using a diamond-coated disc under water cooling. Digital images of each section were captured using a digital camera attached to a stereomicroscope (Nippon SM 225) at a magnification of 8X. Two operators checked each specimen for the presence of dentinal defects (microcracks).

''No defect'' is defined as root dentin devoid of any craze lines or microcracks either at the external surface of the root or at the internal surface of the root canal wall.

''Defect'' is defined if any lines, microcracks, or fractures are present in root dentin.

IV. Results and Statistical Analysis

Determination of the number of teeth having microcracks

Presence of crack --- 1

Absence of crack ----0

No defects in all the three sections of the control group.

Comparison of Microcrack Formation Using Different Rotary File Systems

	HEROSHAPER	HYFLEX CM	XP ENDO SHAPER	CONTROL
CORONAL(9MM)	12	10	3	0
MIDDLE(6MM)	8	6	2	0
APICAL(3MM)	1	1	0	0

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V. Statistical Analysis

Table 1: Descriptive statistics for microcrack formation among different rotary groups

Chi-square Contingency Table Test for Independence				
	HEROSHAPER	HYFLEX CM	XP ENDO SHAPER	Total
CORONAL(9mm)	12	10	3	25
MIDDLE(6mm)	8	6	2	16
APICAL(3mm)	1	1	0	2
Total	21	17	5	43
	.33	chi-square		
	4	df		
	.9879	p-value		

Chi-square Contingency Table Test for Independence					
		HERO SHAPER	HYFLEX CM	XP ENDO SHAPER	Total
CORONAL (9mm)	Observed	12	10	3	25
	% of row	48.0%	40.0%	12.0%	100.0%
	% of column	57.1%	58.8%	60.0%	58.1%
MIDDLE (6mm)	Observed	8	6	2	16
	% of row	50.0%	37.5%	12.5%	100.0%
	% of column	38.1%	35.3%	40.0%	37.2%
APICAL (3mm)	Observed	1	1	0	2
	% of row	50.0%	50.0%	0.0%	100.0%
	% of column	4.8%	5.9%	0.0%	4.7%
Total	Observed	21	17	5	43
	% of row	48.8%	39.5%	11.6%	100.0%
	% of column	100.0%	100.0%	100.0%	100.0%
		.33	chi-square		
		4	Df		
		.9879	p-value		

We examined the association of rotary files with section with the help of the chi-square test, and the chi-square value is 0.33 and p-value (0.9879)(p>5%), which is not statistically significant. There is no association between different rotary files and crack formation.

VI. Discussion

Vertical root fracture (VRF) is one of the most common complications associated with biomechanical root canal preparation, which usually leads to tooth loss. Numerous Ni-Ti instruments with different design were introduced, but all of them causes incomplete cracks or even VRF. Bier et al. suggested that craze lines occurred in 4% to 16%, which may progress into fractures during retreatment procedures or after long-term functional stresses such as chewing. Fractures or craze lines can occur after root canal preparation with NiTi rotary systems and every following additional procedure in endodontics like obturation and retreatment.[11]

The imperative goal in endodontics is resistance to tooth fracture as such fractures might cause a decrease in the long-term survival rate. It is crucial to find out which rotary instrumentation system is safer to use regarding dentinal micro-crack generation.[12]

Many new NiTi rotary instruments have been developed and introduced by various manufacturers in the latest years.[13]

Rotary instrumentation need only less time to prepare root canals compared to hand instrumentation while it results in significantly more rotations of the instruments inside the root canal. It creates more friction between the files and the canal walls.[14] Tip design, cross-section geometry, constant or progressive taper type, variable pitch, and flute form determine the extent of such a defect formation[15]. Canal micro-cracks

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originate inside the root canal, and may or may not reach the external root surface. File design is also likely to affect the shaping forces on the root dentin [16]. Several studies have evaluated the stress applied to dentin and micro-crack formation in the use of rotary systems. However, studies comparing XP EndoShaper, Hyflex CM, and Heroshaper are limited. Thus, the current study assessed and compared dentinal crack formation following the use of this system.

The taper of the preparation and the files could be a contributing factor in the generation of dentinal defects. Wilcox et al. concluded that a root fractures if more root dentin is removed [16]. Hence, in the present study, a uniformed tapered preparation (0.04) was attempted in all groups.

In this study, teeth were sectioned at different levels, which has a significant disadvantage related to the detrimental effect of sectioning procedure. However, in the present study, this might not have been the situation as no microcracks defects were observed in the control group.

The specialty of endodontics has evolved and got revolutionized over the years [17]. The modern endodontic specialty practice has little resemblance to the traditional endodontic practice [18]. Conventional multiple rotary file system were now replaced with single file systems. However, crack formation in the root canal walls is a concern in the use of single - file systems.

In the present study, XP Endo Shaper (single-file system) causes fewer dentinal cracks as compared to multiple rotary file systems (Hero Shaper and Hyflex CM). Similar results were obtained in a study by Ekta et al.[19] As a supposed evolutionary procedural evolvement of multiple file system (Hero Shaper and Hyflex CM), single file system (XP Endo Shaper)facilitate the cleaning and shaping, comprising one single sterile file for root canal shaping (ISO 30 tip and 4% taper). MaxWire® and Booster Tip technologies together make the XP-endo Shaper a “One File Shaper.” It works with continuous rotation. Max wire technology (Martensite-Austenite electropolish-fleX) was used in the manufacture of the files. The metallurgical alloy gives the instrument high flexibility. Its “snake” shape, superelasticity and extreme flexibility combined with continuous rotation at high speed (800 rpm) and minimal torque (1N) ensure: Minimal stress is applied to dentine walls and due to support from the spring action against the walls; minimizes the risk of micro-cracks in the dentine.

In the present study, the use of Hero Shaper files [20] resulted in the highest incidence of defects compared to Hyflex CM and XP Endo Shaper. The dentinal defect that arises in the root during the cleaning and shaping depends on the taper, design, and rotations per minute (rpm) of the instrument. Hero Shaper rotates at a speed of 450 to 600 rpm and torque of 1.2 N. In Hero Shaper, the helical angle of cutting edges varies from tip to the shank and adapted pitch, i.e., the pitch varies according to taper, positive rake angle, large inner core, which tend to engender more stress on the root walls and thereby possess relatively low flexibility. Increased stiffness with less flexibility of the HeroShaper may have contributed to a larger number of defects in the HeroShaper group in the present study.

The use of Hyflex CM produces a lesser number of defects as compared to Hero Shaper. It has a Speed of 500 rpm and a torque of 2.5 N cm. The reason for the lower incidence of cracks in HyFlex CM is due to its 300% more resistance to cyclic fatigue. This control memory metallurgy of the HyFlex CM file makes it more flexible but without the shape memory. Also, it contains a smaller percentage of nickel than other systems. The decrease of nickel content creates a softer metal with lower hardness .It also changes the metal properties, like the thermal changes, which arise during the processing of the HyFlex CM file. It results in a martensitic metal phase [21,22] which is a more flexible form that outcomes in superior elasticity and enhanced resistance to cyclic fatigue[22].

The results of the present study revealed that there was no significant difference between the groups in the formation of dentinal microcracks. Hence, the null hypothesis is accepted.

Cracks in the coronal portion were more than cracks in the apical region, similar to results obtained by Adorno et al. [23] and Liu [24] et al.

All rotary systems used in the study have a similar apical taper design (tip diameter of 0.30 mm), and this could be the reason for the comparable results in the apical third. The slighter incidence of microcrack formation in apical third could be associated with the size and taper of the master apical file. The standardization of speed and torque settings for various file systems is a drawback of the current study. It was tough to homogenize the downward force used throughout every instrumentation.

Gambarini [25] stated that when the torque of an instrument gets beyond a definite limit the risk of intracanal fracture is increased. Even though XP Endo Shaper has a high rotational speed, fewer cracks may be due to its minimal torque.

Some of the defects seen did not connect with the pulp space. During preparation with rotary files, internal stress is generated in the canals. According to Wilcox [27] et al. the stress is transmitted through the dentin towards the surface and often exceeds the force holding the dentin together. Onnink [28] et al. speculated that a fracture contained within the dentin in one section could communicate with the canal space in an adjacent segment.

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We selected mandibular premolars for the study because of the high prevalence of VRF as reported by Tamse[21] et al. Occlusal load on mandibular premolars during chewing is three times as high as the other teeth. Moreover, we selected teeth with only straight root canals thus anatomic complexities were not considered which did not mimic exact clinical presentation.[15]

One of the limitations of this study was the application of elastomeric material to simulate the periodontal ligament.[6] The clinical situation is more complicate because the presence of periodontal ligament influences the distribution of stresses.

All rotary systems used in this in-vitro study created dentinal defects in root canals. Overall, XP Endo Shaper caused the least number of cracks as compared to the other two groups but there is no significant difference among the three groups.

VII. Conclusion

1. Within the limitation of the study, it can be concluded that all the instruments induced dentinal defect irrespective of file design.
2. Control group showed no cracks.
3. Among rotary file systems, a single file system (XP ENDO SHAPER) induced fewer number of defects when compared to multiple file systems even though there was no statistically significant difference.
4. More cracks were noticed in the coronal and middle section as compared to the apical segment with no statistically significant difference.
5. Shaping ability, ability to eliminate smear layer and canal centering ability needs to be further evaluated.

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Rhinosporidiose Endonasale, Une Maladie Rare: Présentation D'un Cas Observé Au Cameroun

By Vouffo F & Kenna E

Abstract- Rhinosporidiosis is a rare disease. The last reported case in Cameroon dates back 34 years. This may give a false impression of the disappearance of this disease from this territory.

We report the case of a 12-year-old female child, brought by her mother, to a consultation at the otolaryngology department of Limbe regional hospital in September 2015, for right nasal obstruction and ipsilateral epistaxis evolving for six months.

The unpermitted physical examination of the right nostril showed a pedicled polyp, which appeared on the underside of the right lower cornea. This outgrowth of rosacea and bleeding easily on contact was painless. Histopathological examination of the surgical excision specimen of this polyp showed that it was rhinosporidiosis.

In short, rhinosporidiosis is still present in Cameroon. It is desirable that specialists in otolaryngology master its clinical characteristics to discuss this diagnosis in consultation, because the quality of effective treatment depends on it.

Keywords: *nose, rhinosporidiosis, cameroon.*

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Rhinosporidiose Endonasale, Une Maladie Rare: Présentation D'un Cas Observé Au Cameroun

Vouffo F [α] & Kenna E [σ]

Résumé- La rhinosporidiose est une maladie rare. Lez dernier cas rapporté au Cameroun remonte à 34 années. Ce qui peut donner une fausse impression de la disparition de cette maladie de ce territoire.

Nous rapportons le cas d'un enfant de sexe féminin, âgé de 12 ans, amené par sa mère, en consultation au service d'otorhinolaryngologie de l'hôpital régional de Limbé en Septembre 2015, pour obstruction nasale droite et épistaxis homolatérale évoluant depuis six mois.

L'examen physique sans préparation de la fosse nasale droite montrait un polype pédiculé appendu à la face inférieure de la cornée inférieure droite. Cette excroissance d'aspect rosacé et saignant facilement au contact était indolore. L'examen histopathologique de la pièce d'exérèse chirurgicale de ce polype a montré qu'il s'agissait de la rhinosporidiose.

En somme, la rhinosporidiose est encore présente au Cameroun. Il est souhaitable que les spécialistes en oto-rhino-laryngologie maitrisent ses caractéristiques cliniques afin d'évoquer ce diagnostic en consultation, car l'efficacité du traitement en dépend.

Mots clés: *nez, rhinosporidiose, cameroun.*

Abstract- Rhinosporidiosis is a rare disease. The last reported case in Cameroon dates back 34 years. This may give a false impression of the disappearance of this disease from this territory.

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Keywords: *nose, rhinosporidiosis, cameroon.*

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I. Introduction

La rhinosporidiose est une maladie mycosique rare, causée par Rhinosporidium seeberi. Elle touche à la fois les hommes et les animaux, notamment les animaux domestiques [1]. L'agent causal vit dans les eaux douces stagnantes et c'est en entrant en contact avec ces eaux qu'on se contamine. Ce germe n'a jamais poussé en culture au laboratoire et présente les caractéristiques morphologiques des champignons et de protozoaires. Mais il est largement considéré comme un champignon ([2]. La contamination directe d'homme à homme n'est pas démontrée et la maladie se manifeste par une lésion polypoïde de localisation le plus souvent endonasale. De rare cas ont été décrire sur la conjonctive, le rhinopharynx, le rectum, le vagin, l'urètre, la bouche et la peau. La rhinosporidiose touche tous les continents et sévi de manière endémique en Inde, au Sri Lanka et en Amérique du sud [1]. Ces pays totalisent à eux seul 85% des cas [3] rapportés. En Afrique et jusqu'en 1990, la majorité des cas rapportés [3] venaient de L'Ouganda (54.8%) et de l'Afrique du sud (18.3%). Le Cameroun et ses pays limitrophes tel que le Congo, le Gabon, le Tchad et le Nigéria n'en sont pas épargnés [2,3].

Les deux premiers cas rapportés au Cameroun remontent à 1976, [4] suivi d'un autre en 1981[5]. De 1981 jusqu'en 2015, soit 34 ans, le Cameroun n'a plus publié de cas. Ce qui peut donner une fausse impression de la disparition de cette maladie de ce pays.

Nous présentons et discutons dans le présent article, un cas de rhinosporidiose endonasale diagnostiqué en 2015 au service d'ORL de l'hôpital régional de Limbé.

II. Observation

Un enfant de sexe féminin, âgé de 12 ans est amené par sa mère, en consultation au service d'oto-rhino-laryngologie de l'hôpital régional de Limbé en Septembre 2015, pour obstruction nasale droite et épistaxis homolatérale évoluant depuis six mois sans aucun traitement antérieur. Aucune notion de baignade dans les eaux stagnantes n'est notée dans les antécédents, et ce d'autant plus que cet enfant est né et grandit dans la ville de Limbé, capital régionale du sud-ouest Cameroun.

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L'examen physique sans préparation de la fosse nasale droite montrait un polype pédiculé appendu à la face inférieure de la cornée inférieure droite. Cette excroissance d'aspect rosacé et saignant facilement au contact était indolore. La narine gauche était libre et le reste de l'examen ORL et général était normal.

Le diagnostic clinique retenu était celui d'une tumeur bénigne de la fosse nasale droite nécessitant une analyse histopathologique de la pièce de biopsie exérèse. A près préparation nasale droite à la xylocaïne naphazozolinée, nous avions noté une réduction spontanée du volume de la masse, ce qui a facilité la résection de la base de son pédicule au bistouri électrique suivi d'une cautérisation hémostatique. Cette masse grossièrement lamellaire a été envoyé dans une solution de formol à 10% laboratoire d'anatomopathologie du centre hospitalier et universitaire de Yaoundé pour une analyse histopathologique qui a été conduite selon les procédures analytiques en vigueur [6].

L'examen macroscopique a montré un fragment tissulaire de couleur rosâtre, mesurant 2.3 cm de grand axe et friable à la coupe. Il a été inclus partiellement dans une histossette et placé en post-fixation dans une solution de formol à 10% pendant douze heures de temps, puis déshydraté dans des solutions d'éthanol de concentration (de 70°-100°). Ensuite il a été clarifié dans trois bacs de xylène suivi d'une imprégnation en paraffine. Après l'enrobage dans la paraffine, cet échantillon a bénéfié d'une coupe au microtome à 4 μ d'épaisseur puis coloré à l'hématoxyline-éosine et lu au microscope optique.

Cette analyse microscopique a montré en surface un épithélium malpighien ou respiratoire focalement hyperplasique sans atypie cytologique. Le chorion est le siège d'un granulome lymphocytaire dense, sans cellules épithélioïdes et au sein duquel on note la présence de nombreux sporanges à paroi épaisse et d'âge différente. Les uns sont intactes et remplis d'endospores. Les autres affichent une ouverture pariétale à travers la quelles les endospores sont libérés dans le tissu de voisinage (fig. 1). Les derniers plus nombreux sont vides et gardent leurs parois épaisses (fig. 2).

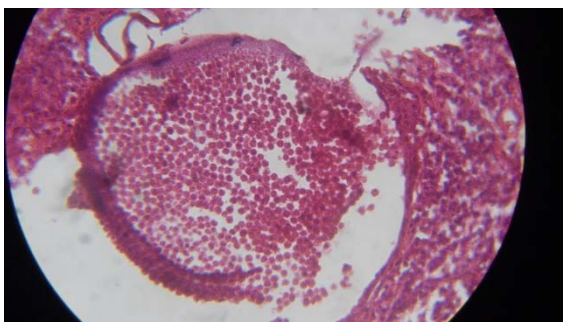


Fig. 1: Sporange libérant les endospores dans le tissu de voisinage (H&E x100)

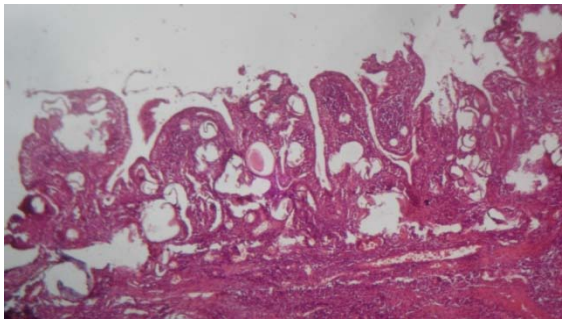


Fig. 2: Sporanges vides dans un tissu densément inflammatoire chronique lymphocytaire (H&E x40)

Fort de ces données clinicopathologiques, le diagnostic de la rhinosporidiose endonasale a été posé. Le patient n'a plus reçu de traitement additionnel et un plan de suivi semestriel mis sur pied. Aucune récidive locale n'a été observée après un recul de trente mois.

III. Discussion

La rhinosporidiose est une infection mycosique rare, touchant à la fois les humains et les animaux domestiques et causée par Rhinosporidium seeberi. Elle est connue depuis environ 100 ans et les premiers cas fut diagnostiqués en Argentine [7].

Rhinosporidium seeberi était au départ classé parmi les champignons compte tenu du fait qu'il est coloré par les colorations fongiques telles que le gomori et le PAS. Par la suite, il a été démontré qu'il présente à la fois les caractéristiques morphologiques des champignons et des protozoaires [8,9]. Plus récemment et sur la base des analyses phylogénétiques, la classe Mesomycetozoa a été retenu dans la taxonomie pour contenir ce germe [7] qui, désormais classé parmi les parasites protozoaires [1,7]. Cette classe possède deux ordres : Dermocystida et Ichthyophonida. Dans l'ordre Dermocystida, on a deux familles parmi lesquelles Rhinosporideaceae qui inclut Rhinosporidium seeberi.

Au plan épidémiologique, il est a noter que la rhinosporidiose est une maladie ubiquitaire. Bien que la majorité des cas soit diagnostiquée en Inde, au Sri Lanka et au Brésil [3], on en rapporte dans tous les continents. En Afrique, toutes les sous-régions sont concernées. Jusqu'en 1990, la majorité des cas rapportés [3] venaient de L'Ouganda (54.8%) et de l'Afrique du sud (18.3]. La maladie touche à la fois les hommes et les femmes avec un ratio femme/homme de 1/5.25 [10]. Le cas que nous rapportons est de sexe féminin. Les raisons de cette forte prévalence de la rhinosporidiose chez l'homme ne sont pas élucidées jusqu'à présent. Certains auteurs suggèrent sans preuve scientifique que cette forte prévalence masculine est liée à l'hyperactivité de l'homme tant pour les travaux champêtres qu'aquatiques. Cela d'autant plus qu'il est établi que Rhinosporidium seeberi vit dans les eaux stagnantes et peut être dans le sol contaminé [11] qui constituent sont habitat naturel. Toutes les tranches

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d'âge sont concernées. Ainsi, on les cas sont rapportés tant chez les enfants [10], les adolescents, les adultes [3,12] et les personnes plus âgées [7]. Notre patiente était un enfant âgé de douze ans. Dans la série brésilienne de Francilio A.A. et al[10], les patients étaient âgés de 7-24 ans avec une moyenne d'âge de 14 ans. Bien que les eaux stagnantes constituent l'habitat naturel de Rhinosporidium seeberi, on ne retrouve pas chez tous les patients les antécédents de contact avec de telles eaux, comme par exemple chez notre patiente qui vit en plein milieu urbain et utilise de l'eau courant distribuée par une société agrée. La rhinosporidiose est une maladie rare, et dont les cas ne sont que rarement retrouvés chez les professionnels du milieu aquatique (pécheurs, riziculteurs…etc.). Ce qui soulève une fois de plus la problématique du mode exact de contamination, surtout que la transmission direct d'homme à homme n'est pas encore démontrée [3]. Il est à signaler que certains cas diagnostiqués sont des cas importés [13].

Il est aujourd'hui admis que l'homme entre en contact avec ce germe en manipulant les eaux stagnantes ou les sols contaminés, mais ne s'infecte qu'à l'occasion d'une rupture des barrières cutanéo-muqueuse et/ou immunitaire. L'expansion de cette infection se fait : soit par auto-inoculation après rupture des sporanges qui libèrent ainsi les endospores ; soit par voie hématogènes conduisant aux métastases voire à une septicémie ; soit pare voie lymphatique ou sexuelle [14]. Ce dernier mode témoigne donc de la possibilité de la transmission directe d'une personne à une autre contrairement à qu'on pensait avant. Au vu de cette pathogénèse on comprend que les localisations peuvent multiples, déterminant ainsi la multiplicité des manifestations cliniques.

La localisation principale de cette maladie est nasale dans 70-92% [3,10] des cas suivi par ordre de fréquence par la muqueuse conjonctivale [15,16]. D'autres localisations ont aussi été rapportées telles que les localisations dans l'oreille externe, la parotide, les os le cavum, le larynx [5], la trachée [17], les organes génitaux, le rectum [7] et la peau. Localisation cutanée peut être primaire [12, 18] ou secondaire. Quelqu'en soit le site de la maladie, le bilan biologique en l'absence d'une maladie concomitante est normal. La rhinosporidiose nasale se manifeste par les épistaxis occasionnelles, la rhinorrhée, le prurit nasal, les éternuements et une sensation de nez bouché. Ces symptômes évoluent depuis quelques mois à plusieurs années, parfois jusqu'à cinq années [7] intéressent la narine gauche dans la majorité des cas [10] sans qu'on ne sache les raisons. Dans les antécédents on retrouve parfois une profession à risque ou une notion de contact avec les eaux stagnantes.

L'examen physique montre une obstruction nasale de degré variable soit par un seul polype pédiculé, soit par plusieurs polypes sessiles ou par la combinaison deux [2]. Contrairement aux polypes inflammatoires qui proviennent du méat moyen, la rhinosporidiose nasale intéresse le plus souvent les narines antérieures, le cornet inférieur, le septum ou le plancher. Ce polype de taille variable, est rose ou rouge violacé, d'aspect framboisé, friable, saigne au contact et couvert de points blancs [3]. La maitrise de ces caractéristiques cliniques de la rhinosporidiose doit élever l'indice de suspicion lors de l'examen physique des polypes nasaux. Le cas que nous rapportons siégeait au niveau du cornet inférieur de la narine droite et évoluait depuis six mois sans antécédents particuliers. Cette masse indolore et rosâtre saignait facilement au contact. Le diagnostic clinique de rhinosporidiose n'a pas été évoqué, car la maladie est rare. Le dernier cas rapporté au Cameroun remonte à 34 ans et donc la maladie est tombée dans l'oubli.

Le diagnostic microbiologique reste impossible car Rhinosporidium seeberi ne pousse pas dans les milieux de culture bactériologique ou mycologique. Ce qui rend difficile tout diagnostic microbiologique et l'identification d'antibiotique efficace contre cette maladie.

Seul l'examen histopathologique de la lésion permet à ce jour de confirmer le diagnostic à partir des coupes histologiques colorées soit à l'hématoxyline et éosine (H&E), au PAS, ou au Gomori methenamine silver (GMS) [2,18]. Nos coupes histologiques étaient colorées à l'hématoxyline et éosine. L'examen au microscope optique montre des lésions élémentaires caractéristiques de la rhinosporidiose [19]. Il s'agit d'un fond inflammatoire chronique dense et lymphocytaire, rappelant ainsi un granulome lymphocytaire. Dans ce fond, sont dispersés des sphérules (sporanges) de 10-200μm de diamètre, à paroi épaisse et contenant ou non des milliers d'endospores mesurant chacun 7-9μm de diamètre lorsqu'ils sont matures. Certains sporanges peuvent présenter une ouverture pariétale à travers laquelle les endospores sont libérés dans le tissu de voisinage. L'épithélium de revêtement superficiel est parfois hyperplasique. Le test à la potasse sur le produit d'apposition ou de cytoponction de la masse permet aussi de mettre en évidence les sporanges et leurs endospores [7,12]. Ce test revêt ainsi une importance particulière en ce sens qu'il peut aider à évoquer le diagnostic en période préopératoire, ce qui permet raffiner le geste chirurgical thérapeutique. En plus il est peu coûteux, facile et rapide.

Le diagnostic différentiel se pose au plan clinique avec toutes les tumeurs et polypes nasosinusiens. D'où l'importance de la maitrise des caractéristiques cliniques de la rhinosporidiose par les spécialistes ORL. Au plan histopathologique, on peut confondre la rhinosporidiose avec la coccidioïdomycose (causée par Coccidioïdes imitis) et qui se caractérise histologiquement [20] par une réaction granulomateuse épithéliogigantocellulaire

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centrée parfois par une nécrose caséeuse. Ses sporanges contiennent des endospores en nombre variable, mais rarement jusqu'à vingt.

Le traitement médical efficace à lui seul contre cette maladie reste controversé. La stratégie thérapeutique la plus utilisée [1, 2, 7, 10,15] consiste en une résection chirurgicale de la base d'implantation du polype suivie d'une cautérisation du tissu de voisinage afin de détruire les sporanges et les endospores résiduels qui pourraient s'y trouver. Ce qui réduire significativement le taux de récidive locale. En plus de ce traitement, certaines auteurs pensent qu'il faut associer pendant plusieurs mois des médicaments antifungiques tels que : Dapsone [7,15], kétoconazole [3, 21] et autres. D'où la nécessité de mener une étude comparative de ces deux stratégies. Notre patiente a bénéficié d'une cautérisation de la base d'implantation de la lésion plutôt dans un but hémostatique, car le diagnostic de la rhinosporidiose n'était pas évoqué en préopératoire. Il n'a bénéficié d'aucun traitement médicamenteux une fois le diagnostic confirmé et il n'ya pas eu de récidive locale après un recul de 2.5 ans.

En conclusion, la rhinosporidiose est une maladie rare et bien présente au Cameroun.

La maitrise des caractéristiques cliniques de sa forme endonasale par les spécialistes ORL est capitale pour évoquer ce diagnostic en consultation. La meilleure stratégie thérapeutique consiste en une résection chirurgicale de la base d'implantation de la masse suivie d'une cautérisation locale.

La pathogénèse de cette maladie n'est pas clairement établie, de même des raisons pour lesquelles les sujets de sexe masculin et la narine gauche sont les plus touchés.

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Caries Inhibition of Pit and Fissure Non Cavitated Lesions in Children by Low-Level Lasers- A Clinical Study

By Lt Col Sonali Sharma, Lt Gen SM Londhe SM, Dr. Mithra N Hegde & Dr. Vandana Sadananda

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Material & Methods: 102 patients of either sex, between age of 6 - 18 years having incipient pit and fissure caries on first or second mandibular molar were selected and its respective contralateral tooth type served as control. Occlusal scanning for both groups was done by LASER fluorescence method (DIAGNOdent) and this was baseline value. Group B (Test) was irradiated with 810 nm AlGaAs low level LASER for 30 seconds followed by application of CPP-ACP F remineralizing paste. In Group a (Control) only remineralization paste was applied. The LASER fluorescence values were recorded after 7 days and the treatment protocol repeated. 18 months follow up at 6 monthly intervals included LASER fluorescence serial scanning. Fall in values from baseline indicates remineralization. Statistical analysis was done.

Keywords: dental caries, non cavitated lesions, remineralization, wattage, time.

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Caries Inhibition of Pit and Fissure Non Cavitated Lesions in Children by Low-Level Lasers– A Clinical Study

Lt Col Sonali Sharma [α], Lt Gen SM Londhe SM, [σ], Dr. Mithra N Hegde [ρ] & Dr. Vandana Sadananda [ω]

Abstract- ***Background:*** The incipient non-cavitated carious lesion are characterized by subsurface dissolution which have potential to remineralize. Traditional remineralization protocols have varying results. An effective remineralizing protocol thus would be one which brings about a change in enamel crystal and makes it more resistant to acidic challenges of oral cavity.

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In vitro studies were conducted on enamel samples prior clinical study to ascertain the optimum wattage and time.

Results: Control group had 14.7 failure rate where as Test group had no failures. The optimal wattage and time of irradiation was 0.5 watts and 30 secs respectively.

Conclusions: LASER irradiation leads to caries inhibition as indicated by fall in LASER fluorescence values after irradiation.

Keywords: dental caries, non cavitated lesions, remineralization, wattage, time.

I. Introduction

Dental caries is a dynamic, progressive disease with varying phases of demineralization and increased remineralization.[1-3] To harness the remineralization potentiality of an incipient non-cavitated lesion, it becomes imperative that carious lesions are detected and diagnosed well in time and evaluated regularly for any change in the lesion activity. The carious process is a continuum disease process and to monitor it at a specific point will give an unfounded, inaccurate and unrealistic result.[1, 4]

Since over a century, fluoride has been the cornerstone caries preventive strategy. Topical fluoride application had varying success rate. Oral fluoride administration in the form of tablets though effective has documented instances of overdose leading to dental fluorosis, nausea, diarrhea, and abdominal cramps.[5, 6] Safe drinking water is a constitutionally guaranteed right in India and millions of rupees are invested in water sanitization, however, centralized systems of water supply and monitoringare ineffective. Inappropriate fluoride levels in drinking water have led to dental and skeletal abnormalities. [7,8]

Thus, an effective preventive regime would be one that brings about a change in enamel crystal and makes it more resistant to acidic challenges of the oral cavity. One such modality is the inclusion of LASERs in the prevention of caries. High powered LASERs are discontinued for caries inhibition owing to their high cost, bulky equipment and lack of any evidence-based therapeutic application.[9,10]Low powered LASER has many soft tissue oriented clinical applications. Today they are being explored as an alternative to high powered LASERs in hard tissue applications.[11]Hence this study has been designed to explore the possibility of using a low-level LASER as caries preventive and inhibitory tool in caries prone population. The findings of the analysis shall help in making standard treatment guidelines, suitable recommendations for caries prevention and inhibition, to benefit children and help in reducing the caries burden.

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II. Material & Method

1. LASER fluorescence device (DIAGNOdent pen 2190 KaVo, Biberach, Germany)
2. LASER - Aluminium Gallium Arsenide (Whitestar TM, Creation, Verona, Italy)
3. Casein Phosphopeptide- Amorphous Calcium Phosphate Fluoride (CPP-ACPF) paste (GC Tooth Mousse)
4. 37 % phosphoric acid gel (Total Etch™– Ivoclar Vivadent AG, Schaan /Liechtenstein)

III. Procedure for Clinical Study

Ethical clearance and informed consent were undertaken. Patients of either sex, between the age of 6 years to 18 years reporting to OPD having initial pit and fissure caries on first or second mandibular molar were included in the study, and its respective contralateral tooth served as control. The sample size was 102 in both groups. Oral prophylaxis was done. Suspected incipient site1 size 0 lesions, as per Mount and Hume classification, were shortlisted. Patients who scored V0, V1 or V2 in Ekstra and criteria for visual scoring for dental caries and 0, 1 or 2 in ICDAS index and R0 or R1 in the Ekstra and criteria for radiographic scoring were included in the study. But the diagnosis was more conclusive by a more sensitive method of caries diagnosis that is the LASER fluorescence method. The LASER fluorescence pen (DIAGNOdent pen 2190, KAVO, Birbech Germany) scanned the area of interest on the tooth surface. A diagnostic readout as per Lussi criteria served as a baseline reading. The LASER fluorescence method values were recorded for both test and control. The test group teeth - (Group B) were irradiated with 810 nm AlGaAsdiode LASER of 0.5 watts (WhitestarTM, Creation, Verona, Italy) for 30 seconds, followed by the application of remineralizing paste CPP-ACP F (GC –Tooth Mousse). The contralateral tooth type served as a control- (Group A), in which, after recording LASER fluorescence device values, only remineralization paste had been applied. (Fig 1 & 2) The values were then noted on the seventh day, the test tooth was irradiated again, and the remineralizing paste was applied. After that, the follow up at six-monthly intervals included serial scanning with LASER fluorescence pen and comparison with baseline reading. The readings determined if caries had been inhibited, arrested, or progressed. A radiograph was then taken to assess if caries has progressed at the end of the study. (Fig3) The results were then tabulated and computed. Statistical analysis was carried out. (Graph 1)

IV. Procedure to Ascertain Optimal Wattage

Thirty freshly extracted teeth, which were caries-free and without any structural defect, had been selected. They were then sectioned mesiodistally to obtain 60 samples, and the tooth section was coated with nail varnish to obtain windows of 3mm X 3mm on the facial surface, which aided in standardizing the sample's dimensions. The samples were divided into ten groups for each power setting which was being evaluated. Each group had six samples each. The power settings range from 0.1 to 1 watts was selected. For each sample, the preoperative value of the exposed tooth section was evaluated by LASER fluorescence method, and this served as control. The tooth section was surface treated with 37% acid etchant gel for 20 seconds. The LASER fluorescence device's values were then noted. Then they were irradiated with aluminium gallium arsenide LASER of 810nm for 30 sec, and each group were then individually evaluated for different power settings power setting from 0.1 watts to 1 watt. The power setting which were evaluated were 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1. After irradiation for 30 seconds, the LASER fluorescence method reading was again noted. The results were then tabulated and computed. Statistical analysis was carried out. (Graph 2)

V. Procedure to Ascertain the Time of Irradiation

Thirty-six samples surfaces were prepared as before and evaluated with a LASER fluorescence device to obtain the baseline values. The samples were then surface treated for twenty sec with 30 % phosphoric acid, to simulate surface demineralization. The LASER fluorescence device was then used to record the values of demineralized samples. The samples were then divided into six groups of six samples each. The sectioned surfaces of the samples were thereafter irradiated with aluminium gallium arsenide LASER of 0.5 watts for 5 secs, 10 secs, 15 secs, 30 secs, 45 secs, and 60 secs, respectively. The LASER fluorescence device recorded values post-irradiation. Statistical analysis was carried out. The exposure time in which the post-irradiation values came closest to preoperative values was then recoded as the optimal time of exposure. (Graph 3)

VI. Summary of Results

The results are being summarized in the graphs (1-3) and tables (1).In this clinical study, the caries preventive and caries inhibitory potentiality of low-level LASERs was evaluated and was compared with the conventional method, i.e, application of remineralization paste. LASER fluorescence was then used for monitoring the effect of the LASER, and remineralizingagent on incipient non-cavitated carious lesions. Based on the result it was finally concluded that the parameters of the low-level LASER of 810 nm, i.e wattage and time, which brings about the optimal results, were 0.5 watts and 30 secs, respectively. In the

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test group, there was a 100 percent success rate. The control group had 15 failures. The results were statistically significant.

VII. Discussion

Dental caries is the most prevalent oral pandemic disease of dental hard tissues. Acevedo et al. observed that 33 percent of children had non-cavitated lesions between age 11-15 years.[12] SK Jain et al. in an epidemiological study, found that currently, there is a change in pattern and trend of dental caries, and thus, there is an increase in the number of non-cavitated lesions as compared to the cavitated carious lesion, and it is more common at the age of 14 years.[13]The tooth which is most prone to dental caries is the first mandibular molar, especially young permanent molars. Hence in this study, the sample population was drawn from six years to eighteen years, and categorically, the first and second molars were included in the study. The sample population was equidistributed amongst both genders. The test and the control are in the same person, and test tooth is the contralateral tooth type of that of the control; thus, making challenges of the oral environment similar.

The performance of caries detection methods will be assessed by considering two fundamental parameters: reproducibility and validity. The visual and tactile technique depends on the clinical acuity of the clinician, and it is not reproducible even for the same clinician leave alone for other observers. 30 to 40% percent of mineral loss must take place before any carious lesion can be detected on radiograph.[14]Hence a sensitive diagnostic adjunct is required. Current diagnostic aids use the alteration in LASER fluorescence, reflectance, electrical conductance or impedance, and ultrasound transmittal properties of enamel concerning demineralization and remineralization during the continuum of a carious lesion over a lengthy period.[14]

Studies by Lussi et al., Novaes TF, Atrill, Fung L, Burin et al., have all inferred that LASER fluorescence methodis a predictive diagnostic tool and is more sensitive than the traditional method of visual and radiographic examination and. It not only detects early and hidden caries but also aids in longitudinal assessment and monitoring of caries activity.[15-19]Hence in this study LASER fluorescence was selected as an assessment tool to study the effect of low-level LASER on the enamel surface.

At the ultrastructural level, the enamel is formed of closely apposition edhydroxylapatite crystals. Outer 50-100 microns of enamel layer are heavily impregnated with fluoride. Below this zone, there is more concentration of carbonate ions and less of fluoride ion concentration. The super saturation at the surface layer chokes out fluoride from permeating into subsurface layers. The incipient carious lesion and non-cavitated lesions are thus characterized by intact surface layer due to more fluoride in the 50-100 microns of the tooth's outer surface and subsurface dissolution due to decreased fluoride ion concentration.[1, 5-8,20]

The advancement of caries may be impeded or foreshortened using risk modifiers. Fluoride, an efficacious therapeutic risk modifier, present in saliva and biofilm does not affect the biofilm formation and sugar metabolism but reduces demineralization by concurrent precipitation of fluoro-hydroxyapatite, a phase more stable than hydroxyapatite at any given pH. Thus, remineralization is a consequence of fluoride application, not an effect of it.[1,5-8,20] Ten cate et al. have inferred that to remineralize subsurface lesions 150microns deep 5000ppm of fluoride is required, which is not practical and excessive doses can lead to toxic side effects and even be fatal.[20]

Hence an alternative remineralizing paste that could substantiate the effect of fluoride is sought. Casein phosphopeptide amorphous calcium phosphate plus fluoride (CPP-ACP F), has been shown to increase fluoride's uptake into plaque and subsurface enamel by providing bio-available calcium ions, phosphate ions and fluoride ions in the correct molar ratio to form fluorapatite.[21] Hence in this study, we have used CPP ACP F as remineralizing paste both in test and control group.

The limitations of remineralizing studies are that most of the evidence-based studies are invitro and is very different when translated to the complex dynamic oral milieu. The other deterrents for remineralization to occur within the body of a subsurface lesion are calcium and phosphate ions must penetrate the surface layer of the enamel. The highly mineralized and charged nature of the surface layer poses a challenge for ion penetration. To infiltrate to subsurface carious layer a very high molar concentration, of approximately 5000 ppm of fluoride ions, are needed. [1, 20]CPP ACP products if, ingested in significant quantities, will cause side effects. The potential risk increases with patients who have allergic diathesis. The effectiveness of CPP ACP in the remineralizing subsurface lesion isquestionable.[5, 7-8, 20-23] The other drawback of incorporating a paste based remineralization strategy is that a regular and repetitive replenishment of paste to overcome the loss of calcium and phosphate ion. Thus, an effective remineralizing protocol would be one that brings about a change in enamel crystal and makes it more resistant to acidic challenges of the oral cavity.

LASERs, since its introduction in the 1960s, have been studied and piloted to bring about caries prevention on inhibition by increasing acid resistance.[9-11]Ana et al deduced that under specific conditions LASER irradiation can change crystallographic properties of apatite crystals and increasing the acid resistance of lased tooth as well as increased fluoride

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uptake.[24] The LASERs which have been investigated in the past for caries inhibition are CO2, Nd:YAG, Er:YAG, ErCr:YSGG. These LASERs had innumerable encumbrances like that of exorbitant price tag, unwieldy, heavy cumbersome and besides, the result obtained was also highly debatable. Additionally, the majority of the experiments were lab-based and further the diode LASER had never been evaluated as a caries inhibitory technique. Clinical studies at best have been pilot studies or short term. [9-11, 24-26]

Sant'anna has conducted an FT Raman spectroscopy study to evaluate low-level diodes as a caries inhibitory tool.[27] Thus, in this clinical study, we have evaluated low-level LASER therapy as a caries inhibitory tool. Comparing test and control it was inferred, to begin with, that the preoperative LASER fluorescence values for all were in the same range. LASER fluorescence values over time have increased in control group thereby indicating demineralization but decreased in test group thereby indicating remineralization, and thus it can be deduced that LASER irradiation brings about changes in the crystallographic properties of enamel apatite and increases the uptake of CPP ACP F paste much more than when the paste is used alone as in control. Thus, our study is confirmation with other reviewed in vitro studies and short term pilot studies. [24-27,29] (Graph 1)

Today, aluminium gallium arsenide LASER is currently being investigated as an alternative to high powered LASERs. The hypothesized mechanism of action is that these wavelengths selectively target and remove carbonate ions from hydroxyapatite crystals which results in increased acid resistance of enamel. Additionally, the altered mineral has greater uptake of topically applied fluoride and leads to remineralization of non-cavitated lesions.[11]Thus, in this study, we have selected low-level LASER to increase the acid resistance and the remineralizing potentiality of incipient non-cavitated lesions.

For any clinical procedure using a LASER, its optical interaction with enamel and dentin must be thoroughly understood. The LASER interaction with dental hard tissues depends upon irradiation parameters such as wavelength, pulsed or continuous emission, pulse duration, repetition rate, beam spot size, and delivery method. All reviewed studies agreed upon the caries inhibitory role of LASERs, but none ascertained the parameters to bring about the desired result. [26-30] Thus, in vitro studies were first conducted to determine an optimum wattage and exposure time. The least amount of deviation there is from control i.e. the closest the LASER fluorescence value to that of the control, the more successful is the treatment. Thus 0.5 watts and 30 secs is the most optimal value as it has shown LASER fluorescence values closest to the corresponding control. The results are statistically significant. (Graph 2& 3)

Another challenge in this study was to determine the sequence of the protocol of LASER and remineralizing paste application. Carounanidy has opined that the LASER activated fluoride uptake method can enhance the remineralization potential of an acid challenged tooth.[3] Further, the scientific world is divided on whether fluoride application should precede or follow LASER irradiation.[28-29] In the present study, on the test group, the fluoride application succeeds the LASER treatment. There has been a fall in the LASER fluorescence values in the test group (Group A)which was irradiated by LASER followed by fluoride application. This decrease in LASER fluorescence numerics was seen throughout the follow-up period. However, in the control group (Group B) in which only CPP ACP F was applied, the digital numerics initially fell and then again increased. (Graph1) Thereby, indicating that LASER irradiation followed by remineralizing paste is more effective than remineralizing paste used alone.

Each study has its limitations, and no research is flawless, nor should it be expected, as is envisaged in this study. The most reliable way to assess the clinical impact of a novel technique is through its endpoint, such as reversal of carious lesion or survival of the tooth after LASER caries inhibition. However, this standard may be impractical for the evaluation of new therapies, because long periods with large sample size are required for these clinical endpoints to be objectively achieved. A surrogate endpoint can expedite the trial process. A surrogate endpoint is identified as a biological marker that is designed to proxy for a clinical termination. Clinical endpoints, on the other hand, are distinct, direct measurements that reflect how a patient responds to treatment. Clinical endpoints are the most credible characteristics used in the assessment of the benefits and risks of a therapeutic intervention. In this study, we have used a clinical endpoint over a long follow up of eighteen months.[30] Since dental caries is a dynamic disease, a surrogate endpoint should be determined to evaluate the validity of this novel technique.

Another limitation is that enamel chemistry studies should also be included to support promising results. The synergism of the low-level LASER and remineralizing paste should be studied at a molecular level.

Thus, this novel concept of combining low-level LASERs with remineralizing paste seems like a promising preventive and inhibitory modality for non-cavitated initial caries provided that they are diagnosed early. For early detection, a LASER fluorescence device is found to be a reliable and accurate tool.

VIII. Conclusion

1. The optimal wattage and the optimal time of irradiation, which could bring about the caries

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inhibitory potentiality of low-level LASERs clinically were 0.5 watts and 30 seconds respectively, in the age group 6 years to 18 years.

2. The test group, in which the teeth had been irradiated with low-level LASERs followed by application of remineralizing paste i.e. CPP ACP F, had a 100 % success rate. The control group, in which only remineralizing gel had been applied, had a failure rate of 14.7 %, which necessitated operative intervention.
3. The low-level LASERs increased the uptake of remineralizing paste in irradiated teeth. Thus, LASERs followed by remineralizing paste seems to be the appropriate sequence to be followed.
4. Remineralizing paste alone did not have sufficient long term caries inhibitory potentiality.
5. The low-level LASERs can be used as a viable option to the conventional method, as an accepted modality in preventive and caries inhibitory regimen of dental caries in routine dental practice.
6. For subsurface incipient carious lesions, the LASER fluorescence device is found to be a reliable and accurate diagnostic tool.

- Conflict of interest statement: The authors declare there has been no conflict of interest.
- Ethical Approval: Institutional Ethical clearance has been obtained.
- Informed Consent: Informed consent was obtained from all individuals participating in the study.

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Table 1

Succes & Failure in Control

	Frequency	Percent
Failure	15	14.7
Success	87	85.3
Total	102	100

Sex	Frequency	Percent
Male	51	50
Female	51	50
Total	102	100

		GENDER		Total	Pearson Chi-Square	p-value
		Male	Female			
Failure	Failure	1	14	15	13.21	<0.001
	Success	50	37	87		
Total		51	51	102		

Failure	N	Mean	Std. Deviation	t-value	p-value
Failure	15	14.4	3.869	1.68	0.096
Success	87	12.7471	3.458		

The percentage of success in control is 85.3 percent and failure is 14.7 percent. The sample population is equally represented by both the genders. Correlation of gender and failure shows that 14 failures are seen in females and 1 failure in males. This inference is statistically significant. Age has no bearing on the outcome. The comparison for test group is not computed as there are no failures in test group.

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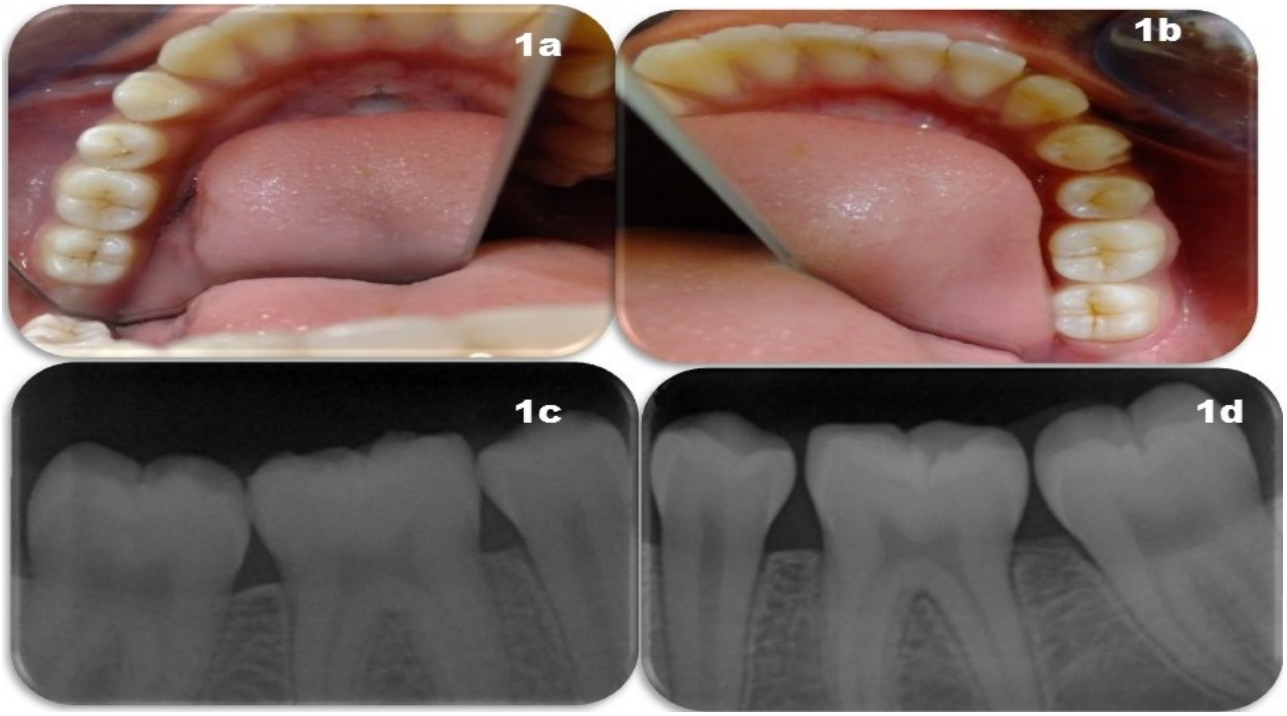


Fig. 1: Preoperative

Fig.1a: Preoperative Clinical Picture of Control 47; Fig.1b: Test 37 Fig.1c: Radiograph Control 47; Fig.1d: Test 37



Fig. 2: Clinical Procedure

Fig 2a: Laser Fluorescence Preoperative Value of 21 in Control 47; Fig 2b: Laser Fluorescence Preoperative Value 26 in Test 37, Fig 2c: Laser Irradiation, Fig 2d: Laser fluorescence value 25 in Test 37, Fig 2e: Remineralizing paste applied

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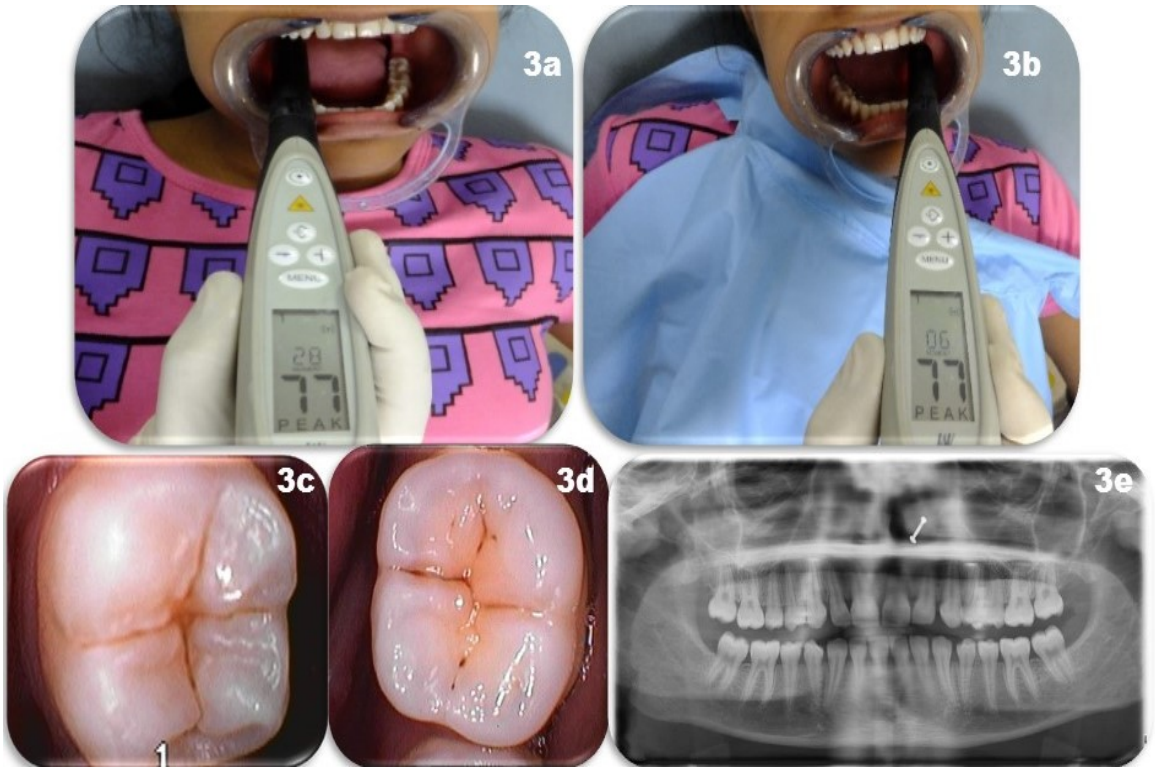
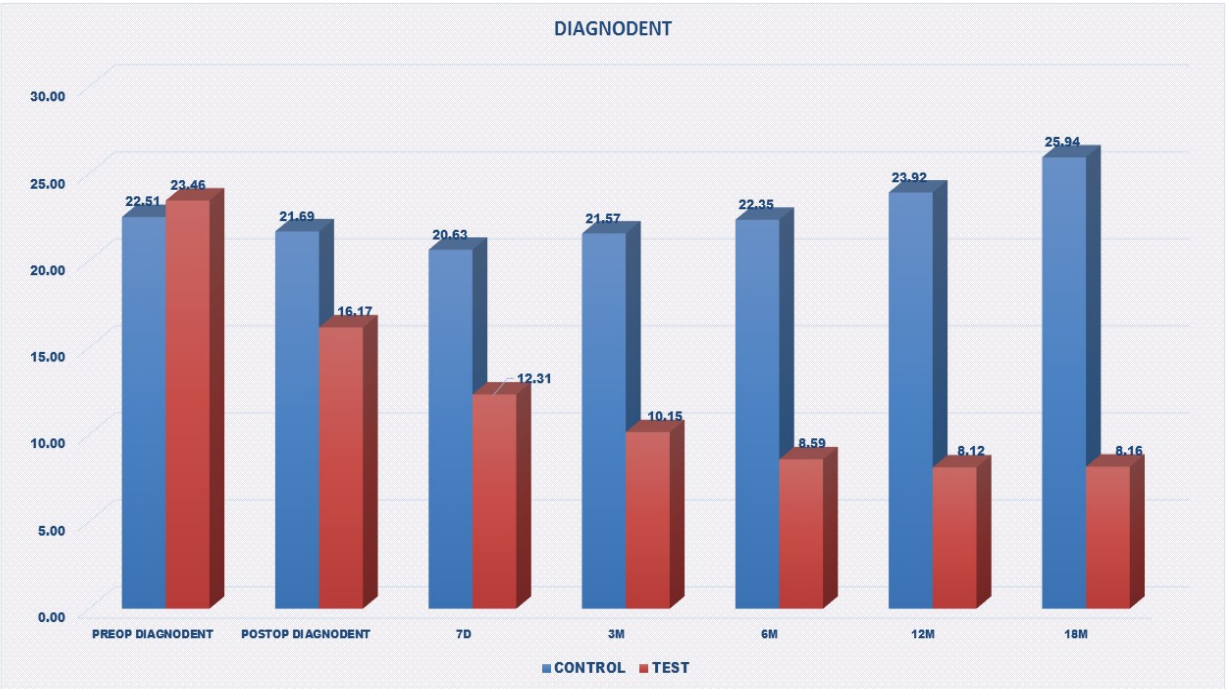


Fig. 3: 18 Month Follow Up
Fig 3a: 18 month follow up Laser Fluorescence Values of Control -28;
Fig 3b: Test- 06; Fig 3c: Tooth no 47; Fig 3d: tooth no 37; Fig 3e: OPG

GRAPH 1

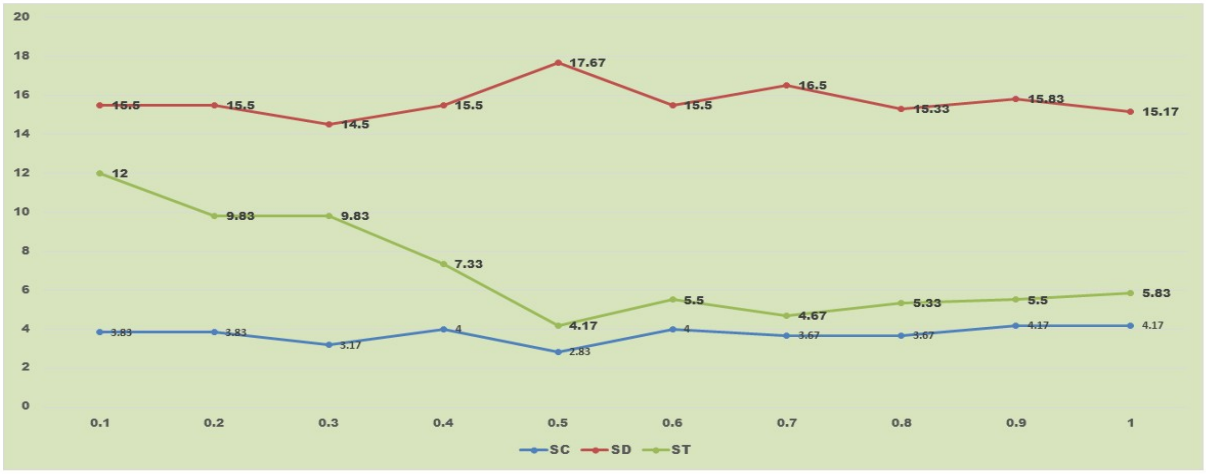


GRAPH 1: Comparison of Laser Fluorescence Values

Laser fluorescence values over time have increased in control group thereby indicating demineralization but decreased in test group thereby indicating remineralization.

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GRAPH 2

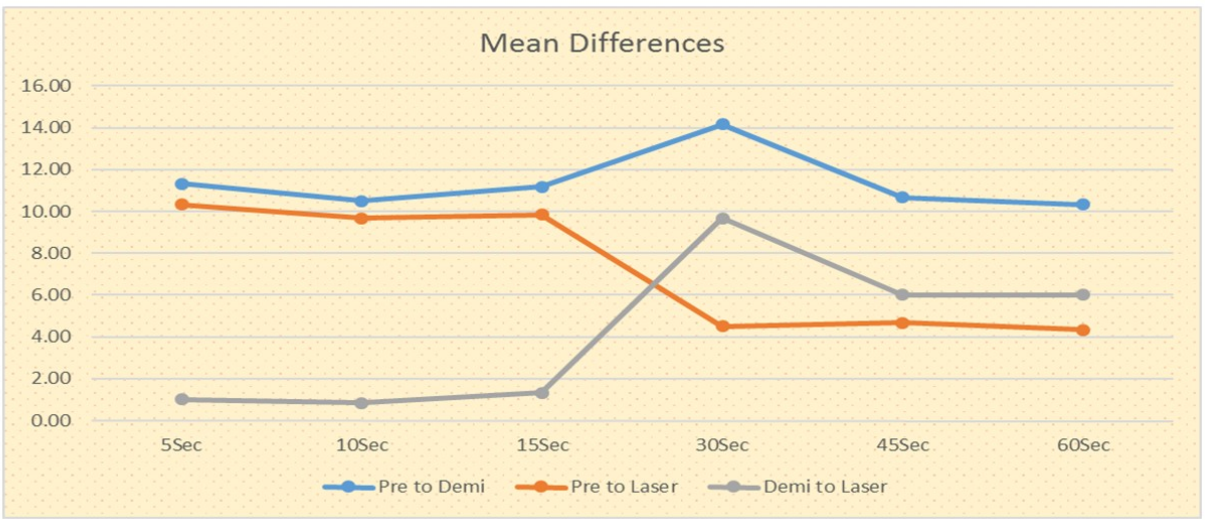


GRAPH 2: Optimum Wattage

There is an inverse correlation between increase in power and laser fluorescence i.e Laser fluorescence method values of irradiated surface. Indicating that the increase in wattage alters the tooth and makes it closer in its configuration in tooth structure to that of control, thus the values of control and treated group i.e laser irradiated by laser. Beyond 0.5 watts there is not much difference between control and laser irradiated values by laser fluorescence

SC Control; SD – Demineralized; ST - Treated

GRAPH 3



GRAPH 3: Optimal Time of Irridiation

There is significant difference between control and demineralized samples and treated and demineralized samples. The treated samples have laser fluorescence values closer to that of control. The time duration of laser irradiation which brings about optimal changes is 30 sec.

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What is the Importance of the Waterproofing Agent on the Surface Properties of Prosthetic Soft Liners?

By Barcellos ASP, Penteado MM, Alvarenga JA, Junqueira JC, Melo RM, Valera MC, Bresciani E & Carvalho RF

São Paulo State University

Summary- *Background:* Prosthetic soft liners are often used in clinical routine in patients requiring temporary rehabilitation.

Objectives: To evaluate the effects of the waterproofing agent on the adhesion of Candida albicans and the superficial properties of prostheticsoft liners, with and without thermopolymerization. Methods: 32 discs of different brands of relining resins (SC and SR) were made. These were randomized into 4 groups (n=8) and submitted to surface roughness tests, profilometry, scanning electron microscopy and goniometry. Another 40 discs of each brand were made to evaluate the adhesion of Candida albicans through the counting of viable cells of Colony-Forming Units (CFU/mL).

Results: The absence of waterproofing liquid caused increased roughness of the material, as well as thermopolymerization, which incorporated pores inside. After 24 hours Candida albicans showed reduced biofilm formation capacity and resin SR with glaze and thermopolymerization showed a reduction in the microorganism count when comparing to the other groups after 24 and 48 hours.

Keywords: *prosthodontics, candida albicans, denture liners.*

GJMR-J Classification: *NLMC Code: WU 500*



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What is the Importance of the Waterproofing Agent on the Surface Properties of Prosthetic Soft Liners?

Barcellos ASP [α], Penteado MM [σ], Alvarenga JA [ρ], Junqueira JC [ω], Melo RM [¥], Valera MC[§], Bresciani E [χ] & Carvalho RF [v]

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Conclusions: The importance of the application of glaze is associated with the quality of the soft relining resins and the copolymerization does not bring benefits according to the applied tests. Given the fact that it is a porous material, the accumulation of microorganisms is assured, therefore the hygiene of the patient is fundamental.

Keywords: prosthodontics, candida albicans, denture liners.

I. Introduction

Despite the change from a curative to a preventive perspective, a large part of the population faces the consequences of more invasive dental treatments.[1] The absence of teeth is still a reality that influences the quality of life and even the individual's systemic conditions.[2] Several scenarios are found according to region, culture and socioeconomic conditions, but caries and periodontal disease are still frequent and often lead to tooth loss.[1,3] In many places, edentulism is still a public health issue.[4,5,6]

Prosthetic soft liners are often used in clinical routine in patients requiring temporary rehabilitation, such as after extraction and implantation of implants.[7] These are indicated mainly in frames with atrophic ridge or sharp bone resorption, bruxism, xerostomia or presence of antagonist with natural teeth.[8] Given the fact that it is a soft material, it provides greater comfort and ease of adaptation with the prosthesis, which is often an unprecedented condition for the patient.[8, 9, 10] However, it is a material susceptible to dimensional changes over time due to the absorption of water and the increase of surface porosity, resulting in the accumulation of microorganisms and loss of adhesiveness to the acrylic resin of the prosthesis base.[10,11,12]

Many studies on the liner behavior are found[11] mainly in relation to porosity[13], biofilm accumulation[12] development of prosthetic stomatitis and angular cheilitis.[14, 15] However, there is no consensus in the literature about the relationship between increased roughness and the presence of *Candida albicans*.[12, 16]

Aiming to provide greater surface smoothness, lower bacterial colonization and microbiological combat, numerous liners advocate the use of a waterproofing agent after the prosthesis has been relined, but there is no literature report on the effect of this clinical stage, which is often neglected.[12, 14]

This study evaluated the effects of the application of the waterproofing agent on the adhesion of *Candida albicans* and the superficial properties of prosthetic soft liners, with and without thermopolymerization. The null hypothesis is that the use of the waterproofing liquid does not interfere with the adhesion of *Candida albicans*, with and without thermopolymerization.

II. Materials and Methodology

a) Preparation of samples

Two products available in the market were selected to perform the research: SC (Soft Confort, Dencril, Pirassununga, São Paulo, Brazil) and SR (Soft

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Rebase, TDV, Pomerode, Santa Catarina, Brazil). Thirty-two discs (N=32) of each material, with a diameter of 12mm and a height of 2mm, were made from progressive waxing. The wax discs were positioned and kept in a refractory coating gypsum (Bellavest SH Bego, Wilcos, Rio de Janeiro, Brazil) until it was firm. The device was taken to the oven for evaporation of wax (EDG3PS, 1800, São Paulo, Brazil). To obtain the samples, the cocoons were filled by the linershandled according to the recommendation of each manufacturer. Another 40 discs (N=40) of each material were made with the same methodology described, but with a diameter of 6mm and a height of 3mm, for the test of microbiological adherence.

b) Division of groups

Samples of each material were randomized into 4 groups, with n=8 for the evaluation of surface properties and n=10 for the microbiological adherence test.

The following groups were obtained: SCI: Soft Confortresin + Waterproofing agent; SC: Soft Comfort resin without Waterproofing agent; SCT: Soft Confortresin without Waterproofing agent, with thermopolymerization and SCIT: Soft Confort resin + Waterproofing agent, with thermopolymerization; SRI: Soft Rebase resin + Waterproofing agent; SR: Soft Rebase resin without Waterproofing agent; SRT: Soft Rebase resin without Waterproofing agent, with thermopolymerization; SRIT: Soft Rebase resin + Waterproofing agent, with thermopolymerization.

The waterproofing agent was applied twice over the entire surface of the sample, for 30 seconds with 30 seconds between the applications, with the aid of microbrush (FGM, Joinville, SC, Brazil).

The thermopolymerizationwas performed by inserting the samples of each group separately in professional wax heater (Mega Bell, São Paulo, Brazil) in water at 100°C for 10 minutes.

c) Characterization

1. Analysis of roughness

Two samples from each group were submitted to surface roughness test (RA). The specimens were coupled to a condensation silicone cushion Profile (Contene, Bonsucesso, Rio de Janeiro, Brazil) handled according to the manufacturer's recommendations and submitted to RA with 3 measurements set with 120° between them, obtaining an average per sample. The Surftest SJ 400 rugosimeter (Mitutoyo America, Aurora, Illinoins, USA) was calibrated with a cut-off measuring filter of 0.8mm, total length recorded of 3.2mm and a scanning speed of 0.5mm/s. All measurements were performed by a single operator. Statistical analysis was performed using the Shapiro-Wilk test and the T-test.

2. Analysis of surface topography by scanning electron microscope

After Bal-Tec SCD 050 metallization (Bal-Tec, Balzers, Liechtenstein, Germany), two samples from each group were submitted to scanning electron microscopy (Fei, Hillsboro, Oregon, USA) at 100x and 2000x magnification, set at low vacuum and 20kV for high resolution images.

3. Analysis of internal porosity by stereomicroscope

In order to verify the internal porosity of the materials, two samples of each group were cut in half and the interior was observed through a Zeiss binocular stereomicroscope (STEMI2000-C, Carl Zeiss do Brasil Ltda., Rio de Janeiro, Brazil) with colored digital camera, resolution of 4.1 megapixels, optical fiber for better illumination of the specimens, images with 20.5x magnification and field of 10.0mm.

4. Analysis of surface tension

Two samples from each group were submitted to the optical tensiometer (TL 1000, Theta Lite Attention, Lichfield, Staffordshire, United Kingdom) through the sessile drop technique. A syringe (# 1001 Gastight Syringes, 1 mL, Hamilton, Reno, NV, USA) with distilled water was coupled to the equipment and a drop thrown on the surface of the samples. During the first second, the drop undergoes the settlement and after 10 seconds, 30 images per second were captured for 20 seconds. Software One Attension (Biolin Scientific, Lichfield, Staffordshire, United Kingdom) was used to calculate surface energy, with 2 measurements per sample.

d) Colonization of microorganism

1. Adherence of *Candida albicans*

The samples were previously disinfected with 70% alcohol and then sterilized by ultraviolet light, 30 minutes on each surface (totaling 60 minutes), through laminar flow (Grupo Veco, Campinas, SP, Brazil). It placed in centrifuge tubes containing 8 mL of Brain Heart Infusion Broth (BHI, Himedia, Mumbai, India) plus 5% sucrose (Labsynth, Diadema, Brazil) and taken to bacteriological incubator B.D.O. (Eletrolab 101M/3, São Paulo, Brazil) at 37°C for 168 hours, observed every 24 hours for analysis of the turbidity of the medium and possible bacterial growth.

The reference strain *Candida albicans* ATCC 18804 was used for the analysis of biofilm formation on the samples. For activation, the microorganism was cultured in culture medium yeast extract, peptone, dextrose (YPD) in bacteriological incubator B.D.O. at 37°C for 24 hours. Subsequently, the microbial cells were centrifuged at 2000xg (approximately 4000 rpm) for 10 min (MPW Centrifuge, Warsaw, Poland), the supernatant was discarded and the sediment suspended in 6mL of sterile saline solution (0.85% NaCl, Labimpex, São Paulo, Brazil). This procedure was

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repeated. The counting of the number of cells of the suspension was performed in a spectrophotometer (B582, Micronal, São Paulo, Brazil), obtaining a concentration of 10^7 microorganisms/mL, corresponding to wavelength 530nm and optical density of 0.381.

The method described by Vilela*et al.* (2012) with some modifications was used for biofilm assembly. Each sample was inserted into each well of a 24-well cell culture plate (KASVI, Curitiba, Brazil), then 100μL of standard *C. albicans* suspension and 2 mL of BHI broth plus 5% sucrose were added. The plate was incubated with 75 rpm agitation (Chemistry ISSO 9001 - Diadema - Brazil) at 37°C for 24 and 48 hours, with exchange of the BHI broth in 24 hours of biofilm formation.

After the incubation period, the samples were placed in centrifuge tubes containing 10mL of sterile physiological solution (0.85% NaCl) and homogenized for 30 seconds using ultrasonic homogenizer (Sonics Vibra Cell, São Paulo, Brazil) with amplification of 25%, potency of 50W, to break down the microbial aggregates.

Serial dilutions in sterile physiological solution (0.85% NaCl) were performed, seeded in Sabouraud Dextrose Agar (Difco, Detroit, USA) and incubated at 37°C for 24 hours for the calculation of Colony-Forming Units (CFU/mL). For the static analysis, they were transformed into logarithm (Figure 3).

III. Results

With the measurements of the surface roughness, it was observed that the absence of glaze or the thermopolymerization treatment increase the roughness of the relining resin, independent of its brand (Table 1).

SEM images show higher surface homogeneity for the groups that underwent waterproofing (SCI, SCIT, SRI and SRIT). In contrast, the groups without waterproofing (SC, SCT, SR and SRT) have significant irregularities. The thermopolymerization did not interfere in the increase of irregularities (Figure 1).

Through the stereomicroscopic analysis, it was possible to observe significant differences in the internal structure, with a higher presence of pores in the resins submitted to thermopolymerization (SCT, SCIT, SRT and SRIT - Figure 2).

Through the analysis of the surface tension, it was possible to observe that the groups with waterproofing agent had a greater wettability (SCI, SCIT, SRI, SRIT) than the groups without waterproofing agent, except for SRT. Thermopolymerization did not interfere in this aspect (Table 2).

In 24 hours of biofilm formation by *C. albicans*, it was possible to observe a significant difference in the capacity of adhesion of the microorganism to the analyzed materials. The groups SC, SCT and SRIT did not form colonies and the Rebase resin showed greater accumulation of microorganisms (Figure 3).After 48 hours, all groups presented colony formation, but statistically significant difference was only between SC and SRIT, SCI and SRT. (Figure 4)

The results of counting of colonies of *Candida albicans* after 48 hours demonstrate that the Soft Confort resin maintains a pattern of accumulation of microorganism independent of the treatment performed. On the other hand, Rebase resin presented totally different behavior. The groups SRI and SRT reacted in a similar fashion. The effect of the reaction of the glaze with the thermopolymerization (SRIT) was similar to the non-glaze (SR) group, demonstrating that the thermopolymerization did not bring any benefit to the system (Table 3).

IV. Discussion

Although RA is lower when there is a thin film of glaze, the accumulation of microorganisms occurred within the first 24 hours of analysis. Possibly this happens due to the manual process of application of the product, which does not count with a standardization and also by the greater wettability found in the analysis of surface tension in the groups with glaze. According to the SEM, the surface of the resin has less defects, however it is not possible to avoid the penetration of fluids only with the layer of waterproofing agent.

The relining resin in its pure state has larger defects visualized in SEM and also higher RA, probably due to the chemical polymerization process inherent to the material. However, this aspect was not relevant in the process of colonization of microorganisms during the initial 24 hours for Soft confort resin (figure 3), which indicates that the composition of the relining material may initially interfere with the microbial adhesion, since comparing the information available on the package insert of the resins shows that there are different types of initiators and catalysts used and also the monomers present in the waterproofing liquid are different: Soft confort uses ethyl methacrylate and Rebase uses polymethyl methacrylate (PMMA).

The group SRIT also did not present microorganisms in 24 hours, in other words, exclusively for this condition the thermo-polymerization caused chemical reactions that were initially favorable, since this behavior did not remain, as demonstrated by the analysis after 48 hours (figure 4). Despite this significant increase in microorganisms, its low incidence or absence in the first 24 hours is an important factor in post-surgical moments, in which the lower number of microorganisms prevents the development of infections. Given the fact that it is a material that is indicated for use during a provisional period of 1 or 2 months, biofilm formation proves the need to make definitive prostheses and the importance of hygiene as maintenance.

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In terms of the accumulation of microorganisms, for both materials there was no statistical difference due to the absence or different treatments: with glaze and thermopolymerization, only thermopolymerization, only glaze. This allows us to affirm that the absence of waterproofing agent will not interfere in this aspect, but among the strategies proposed, the use of glaze is a fast and agile step during the care of the patients and that can contribute exclusively to the comfort during the use of the relined prosthesis, since the RA is lower (Table 1) and that although the wettability is accentuated (Table 2), there is no statistically significant difference in the microbial analysis (Figure 3).

These results corroborate Kutlu*et al.* (2016)[10], which research it has been shown that the aging process does not always increase the surface roughness of the relining materials and that the increase in porosity may not necessarily lead to the development of *Candida albicans* colonies. In addition, it pointed out other factors involved in the emergence of colonies such as: type of polymerization and acrylic resin composition, surface characteristics, hygiene techniques and period of use of the prosthesis.[17]

Another important property for the adhesion of microorganisms is the hydrophilicity or wettability of the material. The higher the capacity to absorb water, the greater the likelihood of the material adhering to microorganisms and of these being able to develop.[18] The water sorption also interferes in the physicochemical properties of the material, causing it to undergo syneresis and imbibition, thus promoting dimensional alterations.[10,11,12,19] As it was possible to observe in this study through the groups without glaze that visually suffered great dimensional alteration in the thermopolymerization, showing that they absorbed a greater amount of water in relation to the groups that had application of glaze.

From the aspect that the prostheses may transform into microbial niches if there is no adequate hygiene,[10, 12] other researches present the insertion of antimicrobial components in the acrylic resin that help to delay or even avoid the development of colonies.[20] Moreover, many methods of hygiene are proposed to avoid deepening of bacterial plaque and consequently the development of prosthetic stomatitis.[13]

In the attempt to improve the performance of the liners, silicone-based materials were created, materials that have greater chemical stability and do not require the clinical application stage of the glaze. In contrast to the two types of resins, despite the use of the waterproofing liquid in traditional liners, the silicone-based materials are more stable.[7, 11, 21]

The use of the results of the present study in order to predict the clinical behavior of prosthetic soft liners should be done with caution, since the buccal environment presents important differences in relation to the environment used in the study and also because it is a cross-sectional study, without long-term follow-up, which may differ in some properties.

It is possible to conclude that the waterproofing liquid is important for the quality of the soft relining resins, avoiding greater distortions by syneresis and imbibition, in addition to possibly favoring the comfort during the use of the relined prosthesis, since it provides greater superficial smoothness.

It was also possible to verify that the copolymerization did not bring benefits to the system and that the accumulation of microorganisms is inevitable regardless of the absence or presence of glaze, therefore the hygiene of the patient is fundamental to avoid prosthetic problems related to the marked colonization of microorganisms.

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Conflict of Interest

The authors declared that there is no conflict of nterest.

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Table 1: Mean values and standard deviation of mean roughness (Ra) and mean distance between the 05 largest peaks and valleys (Rz)

Group	Ra (Mean ± Standard deviation)	Rz (Mean ± Standard deviation)
SCI	0.28 ± 0.078	3.33 ± 1.59
SC	3.06 ± 0.25	22.45 ± 3.17
SCT	3.7 ± 0.38	33.37 ± 11.97
SCIT	0.64 ± 0.44	5.85 ± 6.77
SRI	0.20 ± 0.06	2.08 ± 0.87
SR	0.95 ± 0.27	9.52 ± 4.23
SRT	0.76 ± 0.23	7.48 ± 3.82
SRIT	0.65 ± 0.47	13.22 ± 13.00

Table 2: Mean values and standard deviation of surface tension

Group	Mean ± Standard deviation (mN/m)
SCI	[a] 46.528 ± 35421.15656
SC	[b] 35.562 ± 10147.68942
SCT	[b] 32.842 ± 1142.684558

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SCIT	[a] 57.288 ± 1694.934955
SRI	[a] 41.700 ± 772.1606051
SR	[b] 29.822 ± 3603.416157
SRT	[a] 45.221 ± 1391.586145
SRIT	[a] 40.470 ± 8152.941187

Table 3: Result of counting colonies of *Candida albicans* after 48 hours

Group	Soft Count	TDV Count
Resin + Glaze	Aa	Aa
ResinwithoutGlaze	A a	AB a
ResinwithoutGlaze + Thermopolymerization	A a	A a
Resina + Glaze + Thermopolymerization	A a	B a

Capital letter represents the comparative in the same column, lowercase letter in the same line

Figure legends

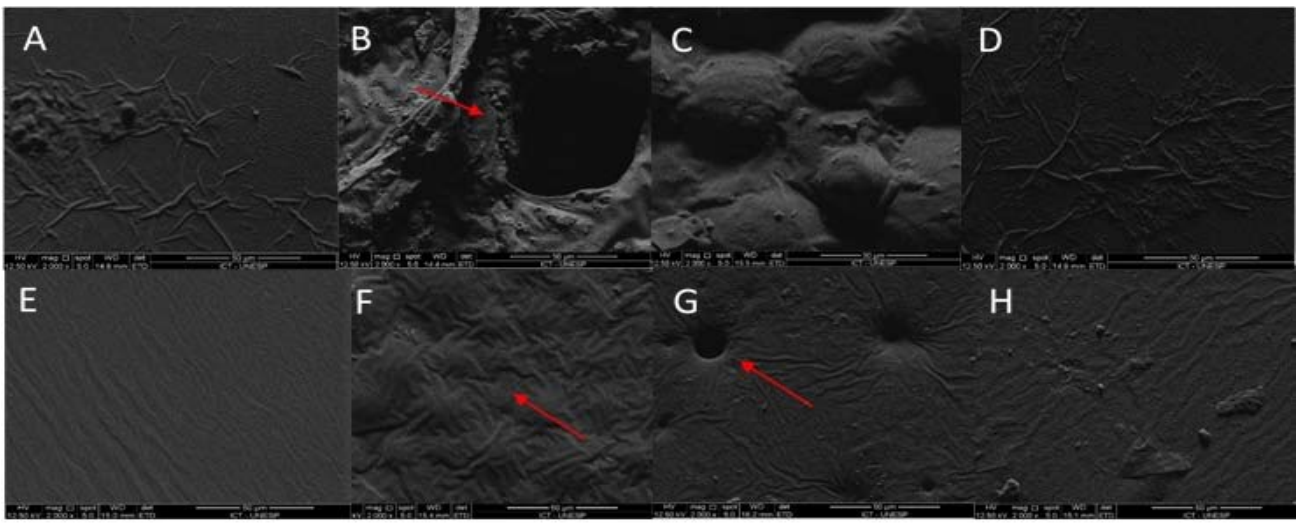


Figure 1: Photomicrograph representative of the surfaces analyzed with 2000x magnification: A-SCI; B-SC; C-SCT; D-SCIT; E-SRI; F-SR; G-SRT; H-SRIT.

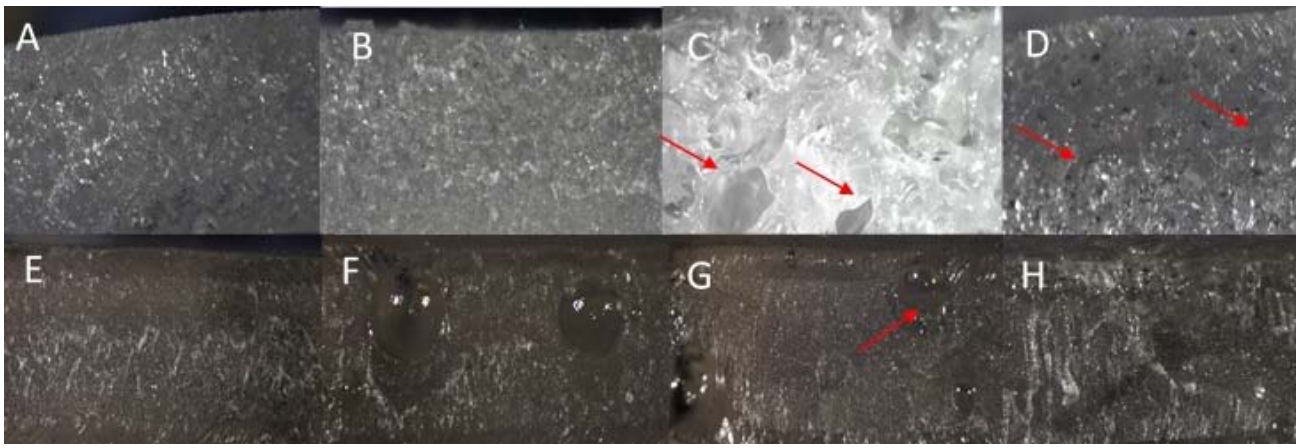


Figure 2: Images representative of sectioned surfaces with 20.5x magnification: A-SCI; B-SC; C-SCT; D-SCIT; E-SRI; F-SR; G-SRT; H-SRIT.

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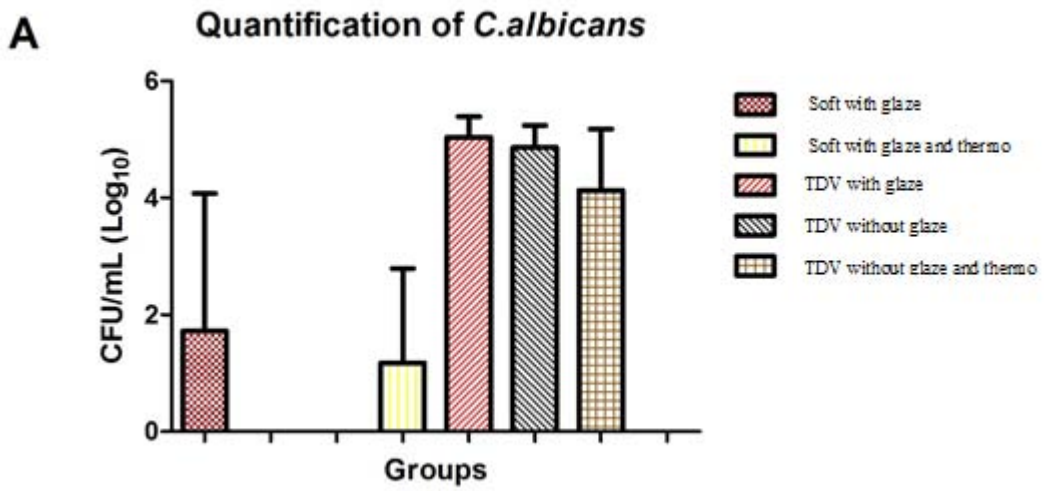


Figure 3: Quantitative analysis of *in vitro* biofilm formation by CFU/mL count: Mean and standard deviation of the number of CFU/mL of *C. albicans* (Log10) for the 24-hour biofilm.

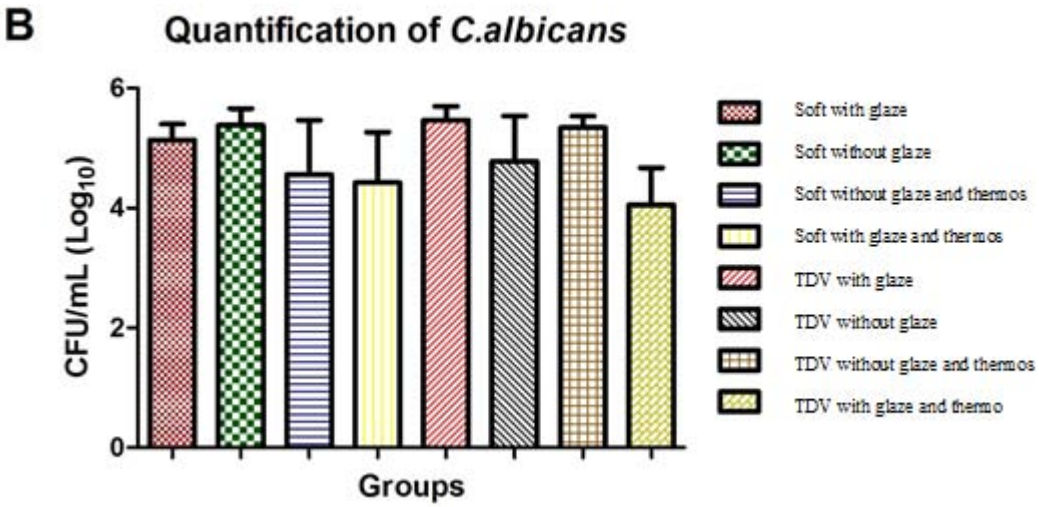


Figure 4: Quantitative analysis of *in vitro* biofilm formation by CFU/mL count: Mean and standard deviation of the number of CFU/mL of *C. albicans* (Log10) for the 48-hour biofilm.

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Preprotective and Protective Protocol in Implantology

By Erza Mulaj, Berat Lenjani, Shqiponje Gashi & Ilirian Lenjani

University Dental Center of Kosovo

Abstract- This presentation, based on various studies and our experiences, aims to highlight the paraprothetic and prosthetic protocol in oral implantlogy. The purpose of this paper is to perform prosthetic simulation prior to any surgical-implant procedure, as oral implant logy arises as a consequence and in response to prosthetics in cases of partial or total insufficiency. By comparing the clinical efficacy of the early and late protocol of dental implant placement according to the protocol. The objectives of this paper are to determine the position, diameter, and number of implants determined by the therapeutic angle that present cases with insufficiency and then indicate the need for prosthetic (suprastructural) work depending on the anatomical considerations of the jaw. Planned prosthetic work may have to vary depending on the appropriate jaw implant position. What needs to be emphasized is proper diagnosis and prosthetic treatment / planning as well as surgical stages.

Keywords: *protocol, prosthetic, prosthetic, implant, oral, surgical.*

GJMR-J Classification: *NLMC Code: WE 172*



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Preprotective and Protective Protocol in Implantology

Cases Praesantation

Erza Mulaj [α], Berat Lenjani [σ], Shqiponje Gashi [ρ] & Ilirian Lenjani [ω]

Abstract- **This presentation, based on various studies and our experiences, aims to highlight the paraprothetic and prosthetic protocol in oral implantlogy. The purpose of this paper is to perform prosthetic simulation prior to any surgical-implant procedure, as oral implant logy arises as a consequence and in response to prosthetics in cases of partial or total insufficiency. By comparing the clinical efficacy of the early and late protocol of dental implant placement according to the protocol. The objectives of this paper are to determine the position, diameter, and number of implants determined by the therapeutic angle that present cases with insufficiency and then indicate the need for prosthetic (suprastructural) work depending on the anatomical considerations of the jaw. Planned prosthetic work may have to vary depending on the appropriate jaw implant position. What needs to be emphasized is proper diagnosis and prosthetic treatment / planning as well as surgical stages.**

Keywords: protocol, prosthetic, prosthetic, implant, oral, surgical.

I. Entry

Over the past decade, implant-prosthetic interventions have become an indispensable part of modern dentistry, helping dentists improve the quality of life of the infants who need dental interventions.

The implantological treatment replaces the missing teeth and should be a paraprosthetic surgery to meet prosthetic needs. Without prosthetic planning, there is no justification for dental implant intervention, and overall prosthetic success depends on the proper protocol and execution of the surgical phase. In many cases, implant therapy is clearly the preferred way to replace missing teeth. Both the surgical and the prosthetic stages require care in planning, diagnostics, evaluation and therapy. (Rizzo 1988)

Maintaining anatomical (natural) structures, functional and aesthetic elements and patient desires are the goals of implant therapy. Implantology treats partial and complete painlessness and as a substitute for other disciplines such as the jaw.

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Diagnosis and treatment planning. Implant therapy is contraindicated in patients with periodontal disease, the first purpose of care being the diagnosis, prevention and treatment of oral diseases. Replacing damaged or destroyed tissue from disease - by prosthetic definition - is the secondary purpose of dentistry (VanBlarcom 1994).

It should be assumed that before any treatment planning process involves implant therapy and is based on the above premise, disease control and prevention are the main planning objectives of treatment (Devan 1952).

The purpose of this illustrated review is to introduce new strategies and developments for the treatment and diagnosis of periimplant diseases. Periimplant disease is a matter of concern for modern dentistry. The number of implants exhibiting biological complications increases as implantable dentistry expands the thinking world. Diagnosing and treating those diseases is still controversial and difficult. We present new treatment for infection control and biological rationale for the additional use of directed bone regeneration, with an illustrative explanation of the treatments presented on two occasions.

The foundation of successful implantological therapy is the diagnosis, treatment planning of both the prosthetic and surgical stages of treatment. The need for proper planning, in other words, cannot be overemphasized. Planning, treatment, and communication with patients should include alternative methods of dental replacement. The principle of informed consent means that the patient is fully informed of all treatment options, including the advantages and disadvantages of each option such as treatment, function, aesthetics and ultimately psychological outcome.

Dental implant therapy is only an option for dental replacement and should be presented as such for all patients. Failure to inform patients of all treatment options is a frequent cause of litigation and malpractice.

II. Epidemiology

The epidemiology of peri-implantitis is heterogeneous due to the different bone loss thresholds and pocket depths used, creating a discrepancy in the

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prevalence figures. The prevalence of peri-implantable mucositis varied between 19 and 65%, while the prevalence of peri-implantitis ranged from 10 to 40%. The prevalence of peripheral implant over a median follow-up of 2 years was 34% at the patient level and 21% at the implant level. Corresponding incidence rates were 0.16 and 0.10 for patient - year and implant - year, respectively.

III. Case Report

Sick N.N. born in 1968 on the basis of general health history (orthopedic intervention in extremities, bone fracture). The patient does not take any medication for any disease, smoker (up to 11 cigarettes per day) with relatively good oral hygiene.

During oral examination, we observed post-canine tooth loss in all four dental arches and after dental examination, implantable prosthetic surgery was required, the second frame we also placed 2 implants at position 25 (3.75X10), 26 (3.75X11.5) and also bypassing at position 24. On both sides of the first superior premolar position, the bone is presented very thin in thickness.



Photo 1: The patient is presented with all the possibilities of interventions to solve the problem of insomnia in both jaws with fixed superficial interventions

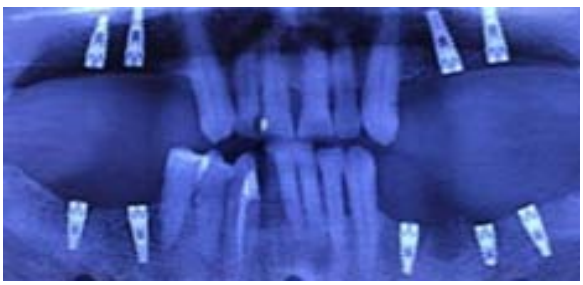


Photo 2: In the first frame we place 2 implants at position 15 (3.3X11.5) and 16 (3.3X11.5) bypassing at position 14 which we will treat prosthetically as a maxillary hanging bridge

To avoid bone regeneration we decided to bypass the area. In the third frame we placed three implants in position 34 (3.3X10.0), 36 (3.75X11.5), 37 (3.75X11.5) where in the next prosthesis the first position 35 will be treated as an intermediate elementn the fourth frame 2 implants were placed in positions 46 (3.3X11.5), 47 (3.3X11.5), prosthetic loading (superstructure) was done after three months starting with implant discovery and healing screw placement.

To avoid any possible movement of the transfer method (which gives the three-dimensional position of the fixation in the patient's mouth) we attach them by means of a curtain thread and place on that thread a pattern that has a very minimal and non-negligible structure.



Photo 3: Superconducting zirconia ceramic material of the upper jaw

The measures deal with polyester material which guarantees the present position at the right time and strength. After the polymerization of the polyether, the masses are sent to the laboratory and the healing screws are placed in the mouth, the pre-prepared lab specimens are placed in the wound and re-applied with the direct abutment provisional method.

After that the protocol is the same as the fixed prosthetics where the structural tests are done based on the work of the fixed prosthetics, biscuit tests, static as well as dynamic occlusion and glazing superiority is cemented.

The periodic examinations that the patient has at our clinic show a clinically good oral and radiologic condition that provides us with a successful implantable and prosthetic prognosis.



Photo 4: Completion of the circular ceramic zircon superstructure in both jaws

It means that in this case pre-prosthetic protocols in implantology have been respected programming of a prosthetic work before implants are placed and co-operation with the laboratory as an important element for a successful rehabilitation of both jaws in both functional and functional terms aesthetic. The position, diameter and number of implants are

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determined by the requirements of the prosthesis and the anatomical considerations of the jaws respectively the jaw bone.

The number, position, and size of dentures will therefore be based on anticipated needs or prosthetic planning, however, rehabilitative prosthetic requirements dictate implant choice and position, in many cases anatomic surgical jaw restraints will also affect the final choice, number and implant placement.

Prosthetic planning or even treatment is the major determining factor in implant, choice, and placement decisions. The functional and aesthetic element of treatment success depends on adequate diagnostics and treatment planning.

IV. Discussion

The use of dental implants to help treat partial and complete edentulism is well documented. However, most implant literature reports results associated with implant survival and success when adherence to fixed placement and loading protocols. Based on conventional research, the elaborated protocols highlight implant osteointegration - 3 months in the mandible and 4 to 6 months in the maxilla. Our work is in line with the papers and literature consulted and develops protocols for clinical procedures for early or immediate restoration or loading of dental implants. The review evaluates factors influencing accelerated loading and restoration decisions, including bone quality and quantity, implant design, implant separation, and prosthetic pattern. Conclusions and recommendations are made based on the consensus group experience in charge of considering these procedures and on the current literature published in these protocols.

V. Conclusion

With reference to the above objectives it is worth noting that the position, diameter and number of implants is determined by the therapeutic angle of the cases with insufficiency and then the need for prosthetic (suprastructural) work is indicated depending on the anatomical considerations of the jaw. The planned prosthetic workings may vary depending on the choice of the appropriate position of the implant in the jaw. Implant prosthetic intervention was used to select partial and total morbidity.

Disclosure

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Iatrogenic and Idiopathic Vocal Cord Paralysis: A Real Obligation of Endoscopic Appraisal

By Delwar AHM, Chowdhury NK, Rahman MS, Khan AM & Hossain ABMT

Abstract- *Background:* The larynx is an inevitable mouthpiece of the human body which has to execute very associated assignment like protection, respiration, and phonation. To fulfill this mission, the larynx should be accommodated, integrated, and sensible. The vocal cord is the concern of larynx, which served all-purpose. If any occurrence due to iatrogenic, idiopathic, or any other causes shut down the vocal cord mobility, the patient is facing miserable complexity in their life.

Methods: It is a cohort retrospective study of 67 cases in the department of otolaryngology and Head-Neck Surgery, Bangladesh, from 01 July 2016 to 31 June 2019.

Results: Incidence among outpatient was 0.06%, inpatient was 0.37%, and total laryngeal disorder, and thyroid operation was 6.51%. Of them unilateral was 64(95.52%) in which both right and left were equal 32(50%) bilateral were 3(2.99%), male were 28(41.79%), and females were 39(58.21%). Amidst them 00-18 years were 2(2.99%), 19-40 years were 21(31.34), 41-60 years 36(53.73%) and above 60 years 8(11.94%), smokers were 34(50.74%), betel leaf chewer 26(38.81%), diabetic were 27(40.30%), hypertensive were 19(28.36%), iatrogenic were 33(49.25%), idiopathic 20(29.85%) and others 14(20.90%), 61(91.05%) treated conservatively, 5(7.46%) surgical, and 1(1.49%) denied the repeated surgery.

Keywords: *vocal cord paralysis (VCPS), magnetic resonance imaging MRI), computed tomography (CT), rigid laryngoscopy(RL), fiberoptic laryngoscopy(FOL), intraoperative neural- monitoring(IONM).*

GJMR-J Classification: *NLMC Code: WO 505*



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Delwar AHM [α], Chowdhury NK [σ], Rahman MS [ρ], Khan AM [ω] & Hossain ABMT [¥]

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Conclusion: Hemiparalysis of the vocal cord may produce many inconsistent symptoms, the surgeon may wait for spontaneous recovery, but bilateral paralysis may need immediate surgical intervention to alleviate respiratory obstacles.

Keywords: vocal cord paralysis (VCPS), magnetic resonance imaging MRI), computed tomography (CT), rigid laryngoscopy(RL), fiberoptic laryngoscopy(FOL), intraoperative neural- monitoring(IONM).

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I. Introduction

The larynx is a winding device of the human body that fabricated voice or speech which are absent in others living being. To generate it, the larynx, particularly vocal cord, should be malleable, organized, and sensible. Phonation is a creation of sound that may be demonstrated by motor activity involves a high orchestration of laryngeal and respiratory neuromuscular regulation.[1] Periaquductal grey matter in the midbrain is an indispensable area for human speech creation.[2] Motor and sensory nerves of the larynx derived from the vagus nerve by way of superior and recurrent laryngeal nerves. The superior laryngeal nerve arises from the inferior ganglion of the vagus and descending to horn of hyoid, where it divided into a small external branches which is only motor supply to the cricothyroid muscle, and a long internal is a sensory supply of larynx above the vocal cord. The recurrent laryngeal nerves in right side leave the vagus nerve by crossing and loops the right subclavian artery and left side crossing the arch of the aorta and running along the inferior thyroid artery in front, behind or between the terminal branches of artery enter into the larynx behind the cricothyroid joint supplies the motor to all muscle of larynx except cricothyroid and sensory below the vocal cord. The superior laryngeal nerve paralysis losses the ability of vocal cord to stretch thyroarytenoid muscle via cricothyroid, consequently recurrent laryngeal nerve unable to tense the other muscle to adduct the cord, therefore it is called adductor paralysis.[3] Due to vocal cord paralysis voice are severely breathy or whispered, hoarseness, reduced loudness and low pitch with possible pitch break.[4] Only recurrent laryngeal nerve lesion where the left side is more than ten times from right side known as abductor paralysis due to contracting, stretching and pulling the-paralyzed vocal cord by cricothyroid muscle towards the midline position consequently the paralyzed abductor muscle are unable to abduct it apart.[5] The most research article showed that most common cause of vocal cord paralysis(VCPS) were iatrogenic.[6] Some reviewers displayed idiopathic was the most common cause of VCPS.[7] Other studies exhibited the common etiological factor was a malignancy.[8] The malignant growth may be created pressure or directly invaded the nerve to generate the VCPS.[9] VCPS is the late symptoms of this diseases of chest, lung, or neck where the surgeon has

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no option to help the patient except waiting for death.[10] Anaplastic thyroid carcinoma also presented with VCPS.[11] In thyroid surgery, the surgeon facing the VCPS when the patient awaking from the anesthesia or the following day when a patient developing hoarseness, dysphonia, dyspnoea, and aspiration.[12] The surgeon is facing the real obligation when the patient is getting the video of FOL or RL. After complete evaluation by history, examination and investigation like x-ray, CT, and MRI of head, neck, and chest showed nothing abnormalities find out then it declared as unknown etiology which solicited as idiopathic.[13] Brain stroke causes further VCPS in which the Broca's area usually the left frontal lobe in the right-handed dominant person of the Brodmann area 44 and 45 ischemia developing expressive aphasia.[14] Accidental causes of VCPS was few might occurred-due to cut-throat injury or road traffic casualty.[15] About 20% of cases of Guillain-Barre syndrome developed respiratory failure and VCPS and required mechanical ventilation after tracheostomy.[16] In developed countries, they are routinely used IONM in thyroid operation to reduce the VCPS, which wasn't possible in the developing countries due to the high cost of the device.[17]

Our aim of the study is to find out the relative incidence and etiological factor of VCPS and pick up the best methods to reduce the calamity of the patient.

II. Methods and Materials

It is a cohort retrospective study of 67 cases in the Department of Otolaryngology and Head-Neck Surgery; Cumilla Medical College, Bangladesh from 01 July 2016 to 31 June 2019. During three years period, outdoor patients was 116128, inpatient was 18268, laryngeal disarray patient was 893, and 136 thyroid operation were performed, and these two were total 1029. Hence the Cardiothoracic and Neurosurgery department were sent 11 laryngeal disorder patients who included in 893 patients. We were performed the endoscopic assessment of 1029 patients by rigid Hopkin's laryngeal telescope. The patient and attendant gave the written informed consent about the examination procedure. Of 893, VCPS patient was 34 in which bilateral paralysis were 02; unilateral was 32 whereas the left-sided were 21 and right was 11. Among 136 thyroid operation, VCPS were 33 whichever 1 was bilateral; unilateral paralysis was 32 in that left-sided was 11 and right-sided was 21. Amidst total 67, bilateral was 3; unilateral was 64, astonishing that right and left-sided were equally 32. 66 patients availed the treatment and 01 patients refused the repeated surgery. The following data collected about the patient: Age, sex, personal habit, past illness, and treatment history and post-operative follow up and complication. All data were calculated using the statistical software of SAS.

III. Results

Incidence among outpatient was 0.06%, inpatient was 0.37% and total laryngeal disorder, and thyroid operation was 6.51% (figure-1). From outpatient laryngeal disarray 893 in which VCPS was 34(4.05%), bilateral paralyses was 2(5.88%), unilateral paralyses was 32(94.12%) considering that left-sided was 21(65.63%) and right was 11(34.37%). From inpatient 136 thyroid operation VCPS was 33(24.26%), bilateral was 1(3.03%), unilateral paralysis were 32(96.97%) since left side was 11(34.37%) and right was 21(65.63%) (Figure-2). The average annual incidence was 22.33. Considering from total, 67 bilateral paralyses was 3(4.48%), unilateral was 64(95.52%), whereas surprising that left and right side were equal 32(50% (Figure-3). Among them, female were 39(58.21%), and males were 28(41.79%) (Figure-3). Age allocated 00-18 years were 2(2.99%), 19-40 years were 21(31.34%), 41-60 years was 36(53.73%) and above 60 years were 08(11.94%) whereas lowest one was 17 years, highest was 75 years although mean age was 43.86 years (Figure-4). Personal habits showed smoker was 34(50.74%), nonsmoker was 33(49.26%), betel leaf and nut chewer was 26(38.81%), and nonchewer was 41(61.19%) (Figure-5). Past illness reviled diabetic was 27(40.30%), hypertensive was 19(28.36%), and both combined diabetes, and hypertensive were 13(19.40)(Figure-5). Amidst them iatrogenic was 33(49.25%), idiopathic was 20(29.85%), brain stroke was 06(8.96%), malignancy was 05(7, 46%) in which bronchogenic carcinoma was 04, and esophageal carcinoma was 01, casualty patient was 02(2.99%), and Guillain-Barre syndrome were 01(1.49%) (Figure-6). Treatment option revealed 61(91.05%) were medical, 05(7.46%) were surgical, and bilateral paralysis 01(1.49%) was refused further surgery with a tracheostomy tube in situ. Of surgical 5, 3(60%) were unilateral paralysis given injection augmentation, and 2(40%) bilateral were treated by cordectomy Figure-7).

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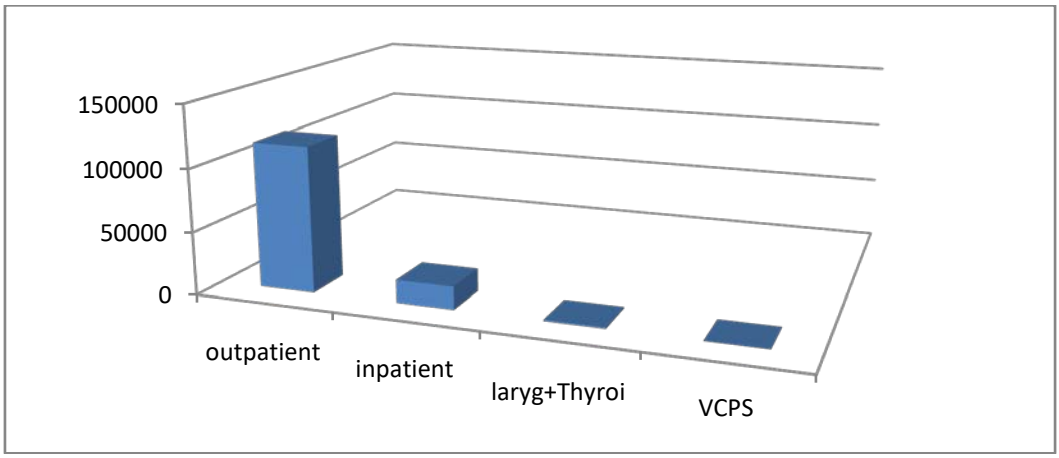


Figure-1: Incidence of VCPS in outpatient, in inpatient and laryngeal disorder+Thyroidectomy patient. [n-116128;VCPS-67(0.06%): inpatient-18268:VCPS-67(0.37%):Larynx+Thyroid-1029:VCPS-67(6.51%)]

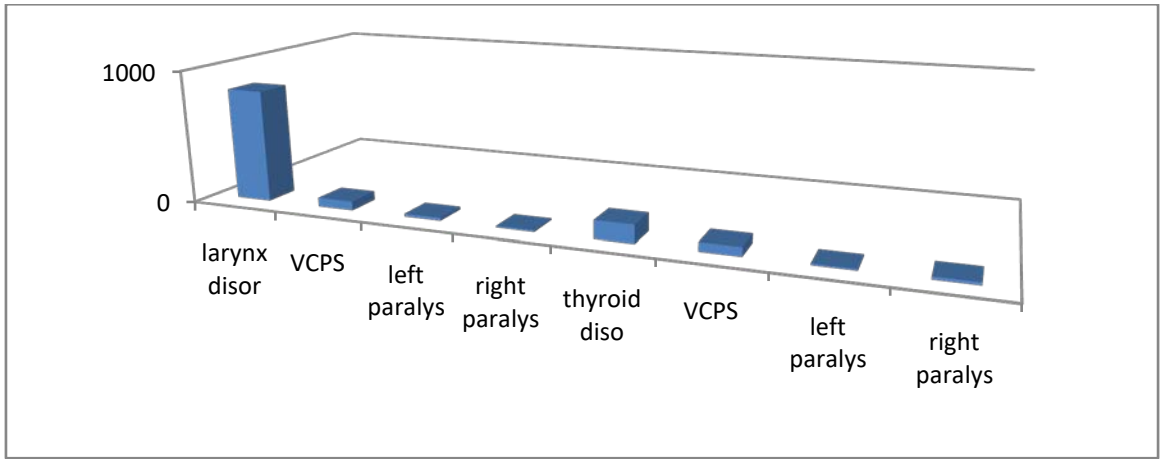


Figure-2: Incidence of laryngeal disorder and thyroidectomy patient independently of VCPS and unilateral paralysis. [{Laryngeal disorders n-839;VCPS-34(4.05%):left paralys-21(65.63%):right paralys 11(34.37%)}{Thyroidectomy n-136;VCPS-33(24.26%):Lt Paral-11(34.37%):Rt paral-21(65.63%)}]

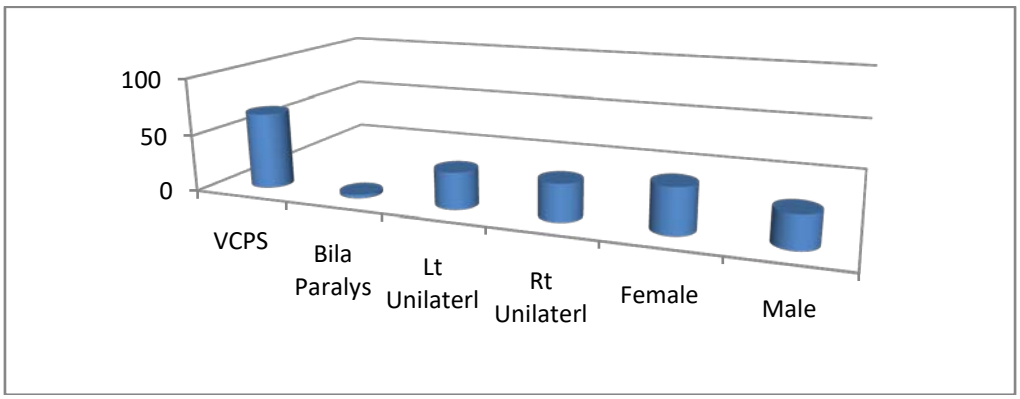


Figure-3: Incidence of type of paralysis and Gender epidemiology. [n-67; Bilateral-3(8.48%): Unilat-64(95.52%): Left Uni-32(50%): Right Uni-32(50%): Female-39(58.21%): Male-28(41.79%)]

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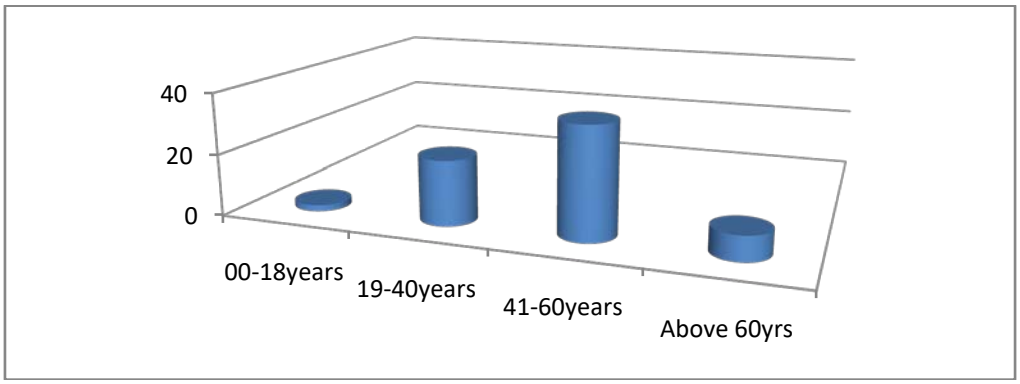


Figure-4: Age allocation.[n-67;0-18yrs-2(2.99%):19-40yrs-21(31.34%):41-60yrs-36(53.73%):Above 60 years-8(11.94%)]

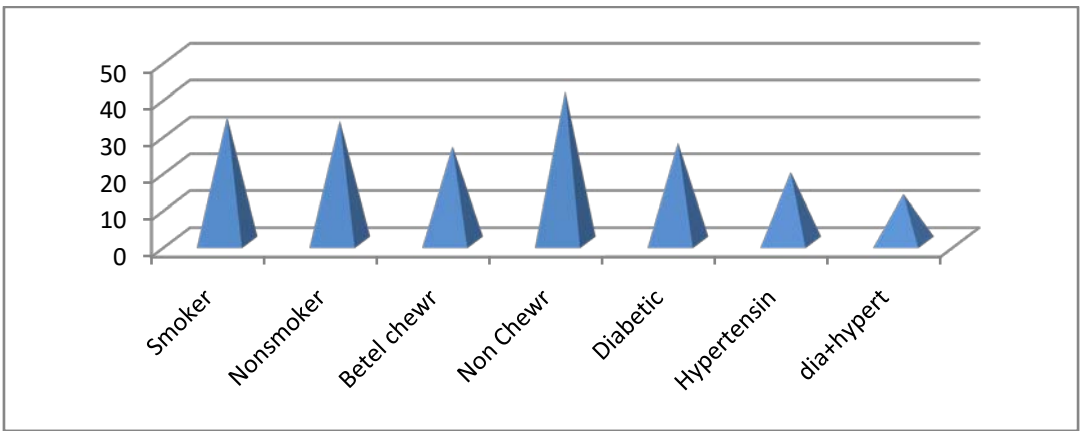


Figure-5: Personal Habit and Past illness. [n-67;smoker-34(50.74%):non smok-33(49.26%):betel leaf chewer-26(38.81%):non chew-41(61.19%):diabe-27(40.30%):hypert-19(28.36%):combined diab+hyper-13(19.40%)].

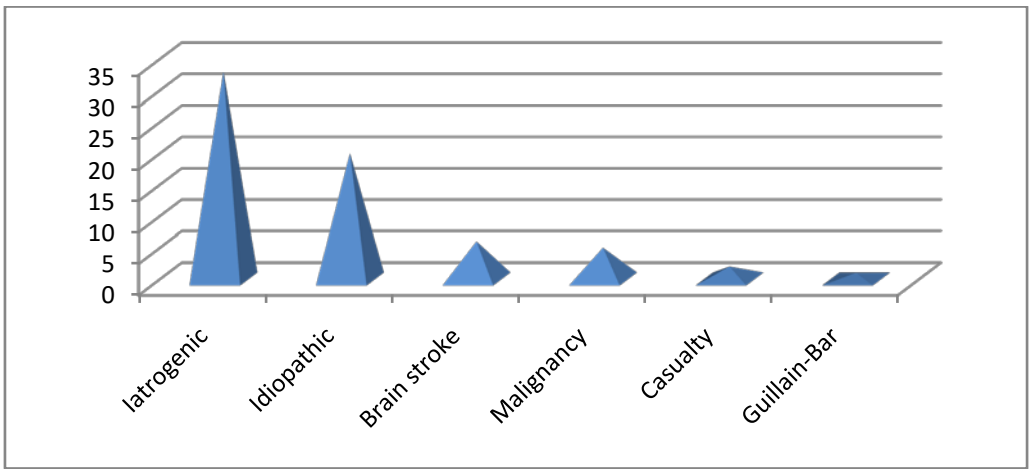


Figure-6: Etiological factor. [n-67; Iatrogenic-33(49.25%):Idiopathic-20(29.85%):Brain stroke-6(8.96%):Malignancy-5(7.46%):Casualty-2(2.99%):Guillain-Barre-1(1.49%)].

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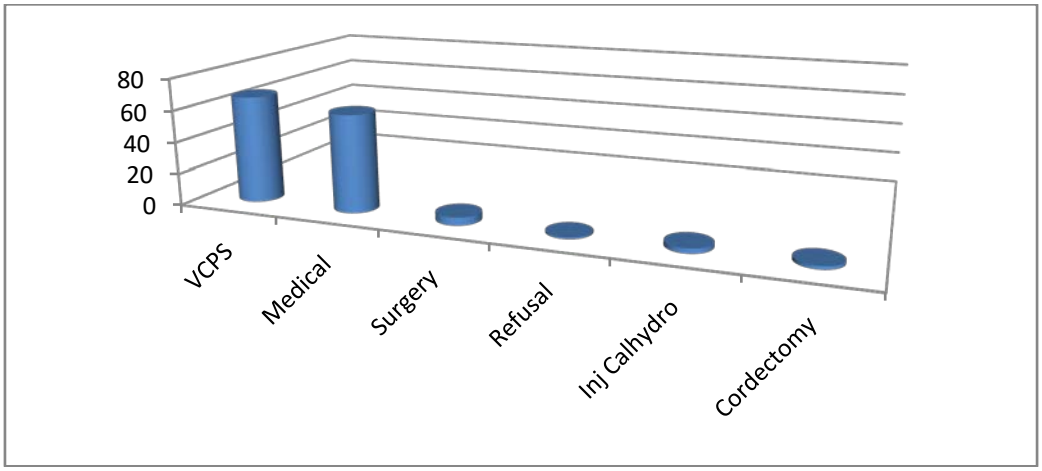


Figure-7: Treatment option.[n-67;Medical-61(91.05%): Sugery-5(7.46%): Refusal-1(1.49%):{Surgery n-5;Inj Calcium hydroxyapatite-3(60%):Cordectomy-2(40%)}]

IV. Discussion

There were so many debated about VCPS in the study to study, posibly due to geographical distribution, developed and developing countries with respect to the availability of latest logistic support for surgical procedure. About patient symptoms, all study showed similarly as hoarseness, dysphonia, reduced pitch, pitch instability, respiratory distress, stridor and aspiration.[18] Incidence of VCPS were variable in a different point of view like 0.06%, 0.37%, 4.05%, 6.51% and 24.26% among outpatient, inpatient, laryngeal disarrayed patient, total laryngeal disorder and thyroid operation operative patients and last of all out of thyroid operative patients. The annual incidence was 22.33 where Rathore NS. et al. was 54, which was more than two times of our study whereas Clerf LH. The study showed 23.15 were hold up our observation.[19, 20] The relative frequency varies considerably due to causative factors variation.[21] Regarding the side of paralysis, unilateral were more than 95% and bilateral less than 5% in our series, which was carried out by Toutoumchi SJS. et al., study.[22] About the unilateral paralysis right and left both sides were equal, but other studies showed the left side was 02-03 times more than the right side.[23,24] In fact in our study left-sided paralysis were 02 times more than right in laryngeal disarray patient, but in thyroid operation, it was reverse the right side was 02 times more than the left.(Total Thyroidectomy-29, Right hemithyroidectomy-72 and left hemithyroidectomy-35), so both sides were equal out of total VCPS. The reality was that most of our thyroidectomy patient were right-sided and IONM device wasn't used in any operation due to cost.[25] In Government hospital, I 99% of patient are poor and able to expenses a maximum of 50-100 USD for their thyroidectomy, whereas one IONM device is more than 300-800 USD. Concerning gender female was 02 times more than male, which was hold up by Rosenthal et al. series.[26] Since Myssiorek D. et al. series displayed males nearly 02 times more than females.[27] Unclear antecedent was due to in our country, female thyroid operation was seven times more than male. About age in our study, 5th and 6th decade showed the highest occurrence, which was reinforced by Gandhi S. et al. series.[28] As regards personal habit and past illness in our study smoker was 34(50.74%), betel leaf and nut chewer was 26(38.81%), diabetic was 27(40.30%) and hypertensive was (28.36%) which was held up by Alassiry H. et al. series.[29] With the reference of our title most common causes were iatrogenic in our study which was thyroidectomy operation was keep up by Ko HC. et al. and Chen HC. et al. series.[30,31] Idiopathic was the second most common cause in our study, whereas Rosenthal LH. et al. series showed it was the third most common cause of VCPS.[26] In our study brain stroke(8.96%) was the third cause of VCPS, which was against the Rubin AD. et al. series.[32] According to UNESCO adult literacy rate of Bangladesh is 72.89%. In our country, one group of people aren't aware of health status, so uncontrolled diabetes, hypertension, unrestrained smoking; betel leaf, and nut chewer were causing brain stroke. Malignancy was the fourth cause in our study, which was also not in our favor whereas Yumoto E. et al. and Rosenthal LH. et al. showed malignancy were the second most common cause of VCPS.[24,26] Casualty was two cases in our study, which was near to Rathor NS. et al. study, they showed, one case.[19] In our study one case of Guillain-Barre syndrome which was supported by Asbury A. K. et al. series.[33] In our study of treatment, 61(91.05%) patients availed medical treatment, 5(7.46%) availed surgery, in which 3(60%) were treated by Inj Calcium Hydroxyapatite for unilateral paralysis, and 2(40%) were treated by cordectomy as sacrificing the voice quality for bilateral VCPS which was reinforced by Jinny Y. et al. and Silva Merea V1. et al. series.[34,35]

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V. Conclusion

The most common causes of VCPS were iatrogenic, and we are concerned it due to the thyroid surgery was done by the Otolaryngologist. It is a real obligation for us. So before starting the thyroid surgery, every surgeon should be adopted with the surgical anatomy. The latest technology should be provided by the Government for poor people. Before telling the patienst idiopathic, a thorough and unified evaluation should be completed about VCPS patient. Now time is demanding a competent surgeon and latest technical support to reduce the catastrophe of the patient.

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Early Childhood Caries and its Association with Socio-Behavioural and Parental Factors among 2-6 Year Old Children in Faridabad

By Dr. Pratibha Taneja, Dr. C M Marya, Dr. Ruchi Nagpal & Dr. Sakshi Kataria

Abstract- The aim of the present survey was to assess the prevalence of ECC and its association with socio-behavioural and parental factors among 2-6 year old children in Faridabad.

Material and method: Risk factors evaluated were: Parent related variables which include family income, educational level, oral health belief, and oral hygiene practices of parents. Secondly, Child related variables which include sex, age, oral health practices, dental visit pattern, deleterious oral habits, feeding and dietary practices of child. Ten kindergarten schools in Faridabad were included and data of 750 children of age group (2 - 6) years was collected. A structured questionnaire was sent to the parents before conducting the study. A prior consent for dental examination was taken from the parents. Dentition status and oral hygiene status (visible plaque) was evaluated.

Results: Chi square tests were applied for evaluation of risk factors. The prevalence of ECC was found out to be 28.8%.

Conclusion: Presence of visible plaque, habit of breast feeding, bottle feeding, frequency and direction of cleaning was found to be the determinants of ECC.

Keywords: *early childhood caries (ECC), visible plaque, pre-schoolers.*

GJMR-J Classification: *NLMC Code: WI 200*

EARLYCHILDHOODCARIESANDITSASSOCIATIONWITHSOCIOBEHAVIOURALANDPARENTALFACTORSAMONG26YEAROLDCHILDRENINFARIDABAD

Strictly as per the compliance and regulations of:

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Early Childhood Caries and its Association with Socio-Behavioural and Parental Factors among 2-6 Year Old Children in Faridabad

Dr. Pratibha Taneja [α], Dr. C M Marya [σ], Dr. Ruchi Nagpal [ρ] & Dr. Sakshi Kataria [ω]

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Keywords: early childhood caries (ECC), visible plaque, pre-schoolers.

I. Introduction

Dental caries is an epidemic disease affecting humans of all ages in regions of most common disease of children. Among all the dental diseases, the prevalence of dental caries in India is increasing and is referred to as "Disease of civilization."[1] According to Centre for Disease Control & Prevention dental caries is perhaps the most prevalent infectious disease.[2] Although it is well understood and preventable but still a global problem among children and young adults. More than 40% of children have caries by the time they reach kindergarten.[3]

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Early childhood caries (ECC) is a serious oral health problem. According to Academy of Pediatric Dentisrty (AAPD), Early Childhood Caries is defined as the presence of one or more decayed (non-cavitated or cavitated lesions), missing (due to caries) or filled tooth surfaces in any primary tooth in a child with 71 months of age or younger. It also has a complex etiology and is considered to be a multi-factorial disease and these factors includes a susceptible host, fermentable carbohydrate diet, presence of dental plaque, high number of cariogenic micro-organisms such as Mutans streptococci, lactobacillus and time factor.[4]

Early Childhood Caries can be a particularly virulent form of caries, beginning soon after tooth eruption, developing on smooth surfaces, progressing rapidly and having a lasting detrimental impact on the dentition.[4]

ECC has also been described as a social, political, behavioural, medical, psychological, economical and dental problem, because it is epidemic in disadvantaged children, regardless of race, ethnicity or culture. Long term follow up reveals that children who experience ECC are more likely to develop dental problems as they grow older and the prevalence rate ranging from 1% to 12% in pre-schoolers of developed countries9 and from 50% to 80% in high-risk groups.[6]

Studies have found that the frequency of ECC is greater among children who fall asleep sipping sugar containing fluid, children in families with a larger number of siblings and those whose mothers are younger.[6] ECC has also being associated with demographic characteristics, parental attitude, parents with low level of formal education or insufficient knowledge regarding oral health and lower household income.[7]

Detailed information regarding the prevalence and influencing factors of ECC provides a valuable tool in the planning, implementation and evaluation of oral health promotion programs. Despite several studies being done on ECC worldwide, there is a paucity of data on the prevalence of early childhood caries7 and its association with Socio-behavioural and Parental factors in Indian context, therefore an attempt is made to assess the prevalence of Early Childhood Caries and its association with Socio-behavioural and Parental factors among 2-6 year old children in Faridabad.

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II. Material and Methods

A cross-sectional analytical study was carried out to assess the prevalence and severity of ECC and its association with socio-behavioural and parental factors among 2-6 year old children in Faridabad. The study population was selected from the 2-6 year old children attending kindergartens, playschools of Faridabad.

a) Inclusion criteria

- 2-6 year old children who were attending preschools, kindergartens of Faridabad.
- Children whose parents gave complete information as per the proforma.

b) Exclusion criteria

- Those children whose parents did not give consent for oral health examination of their child.
- Children who were not willing for clinical oral examination.
- Children who had major systemic disease, craniofacial deformities, syndromes or those who are on long term medication.

III. Sampling

Required study sample was obtained through cluster sampling technique with kindergartens or playschool as sampling unit.

Sample size was calculated using Open Epi Software (version 3). Based on estimated caries prevalence of 42% (using data from pilot study), 5% level of precision, 95% confidence level and a design effect of 2, minimum sample size was calculated as 749 and was rounded off as 750.

IV. Study Variables

1. Independent variables – Independent variables involved in the present study are of two types. Firstly, Parent related variables which include family income, educational level, oral health belief, and oral hygiene practices of parents. Secondly, Child related variables which include sex, age, oral health practices, dental visit pattern, deleterious oral habits, feeding and dietary practices of child.
2. Dependent variables - Prevalence and severity of ECC.

V. Method of Data Collection

Data was collected by a combination of structured questionnaire filled by the parents and clinical examination performed on their children. It was recorded on proforma specially designed for this study.

a) Questionnaire Administration

Children's parents were asked to fill a questionnaire regarding their income, occupation, level of education, oral health belief and oral hygiene habits. It also included questions regarding their child's oral hygiene practices, dietary and feeding habits, dental visit pattern, and deleterious oral habits.

b) Clinical Examination

Oral examination was conducted to examine the Dentition Status and Dental plaque status. The Dentition status will be assessed using Dentition Status Index by tooth surface (WHO criteria, 2013)[8] and Dental Plaque status will be assessed using Visible Plaque Index (Ainamo & Bay, 1975).[9]

A single examiner, assisted by a trained recording person conducted all the examinations. The calibration of the investigator was done prior to the pilot study under the guidance of faulty members in the Department of Public Health Dentistry of the institution. The examiner conducted ADA recommended Type III clinical examination with the aid of plain mouth mirror, and Community Periodontal Index probe. All bio-security measures were strictly followed.

VI. Statistical Analysis

The data collected was entered in the excel sheet using Microsoft Excel Software by the examiner. Then this data was transferred to Statistical Package for Social Sciences (SPSS) version 21, IBM Inc. for analysis. It was subjected to descriptive statistics for calculation of mean, standard deviation, absolute and relative frequencies. Presentation of data was done using Tables and Graphs. Normality of data related to interval or ratio variables was checked by Shapiro Wilk test. As data failed to follow the normal distribution, non parametric tests of significance were used. Mann Whitney U test was used for comparing the mean between two groups and Kruskal Walli's test was used to compare the means of more than two groups. Chi-square test was used for categorical variables. A level of significance was set at 0.05.

Permission from the concerned authorities of the selected schools, preschools, and kindergarten was taken prior to the study. Information sheet in English andin Hindi was provided to the parent of every subject before taking informed consent from them.

VII. Results

a) Demographic Details

Among 277 subjects belonging to 5-6 years age group, 115 (41.5%) were males and 162 (58.5%) were females. *(Table1)*

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Table 1: Gender wise distribution of subjects among different age groups

AGE GROUPS	Males		Females		Total		Pa value
	N	%	N	%	N	%	
2-4 Years	207	43.8	266	56.2	473	100	0.549
5-6 Years	115	41.5	162	58.5	277	100	
TOTAL	322	42.9	428	57.1	750	100	

a Chi Square test

b) Dentiton Status

The Decayed teeth were present among 216 (28.8%) subjects. Although prevalence of decayed teeth decreased from 2-6 year old groups but these differences failed to reach the level of statistical significance ($p>0.05$). Among 5-6 year age group prevalence of Filled teeth with decay was high whereas among 2-4 years age group prevalence of Filled without decay was high($p<0.05$). Prevalence of Missing teeth due to caries across different age groups and revealed that children in 2-4 year age group were having significantly more number of missing teeth due to caries ($p<0.05$) (Table 2).

Table 2: Age group wise distribution of the study population according to dentition status

Age group	Decayed		Filled with decay		Filled without decay		Missing due to caries		dmft		Pit and fissure sealents		Prosthesis/crowns	
	N	%	N	%	n	%	n	%	N	%	n	%	n	%
2-4 years	143	30.2	5	1.1	13	2.7	24	5.1	156	33.0	0	0	0	0
5-6 years	73	26.4	9	3.2	0	0	6	2.2	74	26.7	0	0	0	0
Total	216	28.8	14	1.9	13	1.7	30	4.0	230	30.7	0	0	0	0
Pa value	0.258		0.032*		0.005*		0.05*		0.072		0.00		0.00	

*a Chi Square test, *significance at $p\leq0.05$*

c) Socio Behavioural and Parental Factors & Ecc

Mean number of decayed surface (ds) of children whose parents were either illiterate or were educated upto primary level was significantly more than that who were educated upto middle school, high school, intermediate or graduation. (Table 3).When comparison of mean number of decayed surfaces was done across different occupations, an overall statistically significant difference was not found. Mean number of decayed surfaces for children without visible plaque was found to be significantly lesser than children with visible plaque. Importance and frequency of brushing was not found to be significantly associated.

Mean ds of the children whose parents used finger/toothbrush was more when compared with the parents who used other aids of cleaning but this difference again failed to reach the level of statistical significance. Frequency of dental visit and habit of breastfeeding/bottle feeding showed an overall statistically significant difference with ds.

Supervised brushing and age at which children started cleaning their teeth was not found to be significantly associated with ds.

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Table 3: Socio behavioural and parental factors & ECC

Father's education	N	Number of decayed surfaces among subjects		P value	Post hoc comparison
		Mean	Std. Deviation		
Professional or honours	1	0.00	0.00.	0.020*	6,7>5,4,3,2
Graduate or Post Graduate	24	0.16	0.48		
Intermediate or post high school diploma	8	0.50	0.53		
High school	33	0.24	0.61		
Middle School	107	0.44	1.08		
Primary (upto 5th)	337	0.57	1.02		
Illiterate	123	0.53	1.58		
Father's occupation (N=659)					
Professional	16	0.56	1.50	0.396	
Semi-professional	21	0.09	0.30		
Clerk, farmer, shop owner	19	0.47	0.69		
Skilled	78	0.60	1.34		
Semi-skilled	29	0.44	1.12		
Unskilled	496	0.48	0.98		
Visible plaque					
Absent	366	0.23	0.55	<0.001*	
Present	384	0.78	1.45		
Parental opinion about the importance of brushing (N=748)					
Its important	743	0.51	1.14	0.521	
Its not important	5	0.80	1.30		
Parental tooth paste use					
Yes	702	0.53	1.16	0.204	
No	43	0.27	0.59		
Parental use of toothpaste containing fluoride (N=734)					
Yes	446	0.49	1.04	0.521	
No	60	0.51	1.15		
Don't know	228	0.53	1.30		
Parental frequency of brushing (N=739)					
Once	595	0.52	1.21	0.370	
Twice	134	0.50	0.82		
Sometimes	7	0.42	0.53		
Never	3	0.00	0.00		
Aids used for cleaning teeth by parents (N=746)					
Tooth brush	727	0.51	1.14	0.364	
Dental floss	2	0.00	0.00		
Chewstick/ Miswak	12	0.33	0.65		
Finger	5	1.00	1.22		
Frequency of child's dental visit during past 12 months(N=741)					
Once	475	0.48	1.10	0.009*	2>1
Twice	109	0.60	0.99		
Three times or more	12	0.16	0.38		
Never	145	0.58	1.40		
Age at which child started cleaning teeth (N=750)					
With in 1 year	351	0.64	1.26	0.11	
Above 1 year	399	0.39	1.00		
Who cleans child teeth (N=732)					
Child himself	351	0.64	1.26	0.056	
With parent's assistance	292	0.35	0.69		
Under parent's supervision	89	0.56	1.69		
Direction of tooth brushing (N=733)					
Horizontal	352	0.70	1.43	0.000*	1,3>2,4
Vertical	68	0.26	0.80		
Circular	143	0.49	0.82		

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Don't know	170	0.25	0.65		
Presence of breastfeeding					
Yes	672	0.46	1.00	0.001*	
No	70	0.87	1.95		
Duration of breastfeeding					
6 months	415	0.55	1.09	0.003*	1,2>3
1 year	120	0.70	1.61		1>4
2 year	146	0.27	0.70		
Three years or more	45	0.24	0.52		
Presence of bottle feeding (N=719)					
Yes	610	0.62	1.19	0.016*	
No	109	0.50	0.94		
Duration of bottle feeding					
6 months	288	0.61	1.19	<0.0001*	1,3>2,4
1 year	114	0.33	1.42		
2 year	156	0.56	1.00		
Three years or more	88	0.34	1.03		

VIII. Discussion

In the present study, feeding and oral hygiene habits and practices of the children along with the parental attitude towards oral health were determined.

a) Early Childhood Caries

Studies have found that ECC can have an overall negative effect on the oral health related quality of life of pre-schoolers. Major risk factors for ECC are minority racial status, poor access to dental care, ethnicity, number of siblings, high intake of carbohydrate snacks and parents poor knowledge about the importance of oral health. Pre-schoolers affected by ECC tend to grow slower than caries free children, may be underweight due to difficulty in eating and more likely to have dental problems as adults.

b) Sociodemographic variables

Age profile of the study population: This study population has been compared to other studies such asdone by *Nobile et al*[10], among 36-71 months old children of thirteen kindergarten of Italy, *Azevedo et al*[11], among 36-71 months old Brazillian preschool children.

Father's educational level: Children whose parents were having higher education had significantly lower mean number of decayed surfaces compared to those children whose parents had lower education. This is in accordance with the study conducted by Eronat et al[12] whereas Szatko and coworkers[13] found a strong independence of parent's educational level with their level of knowledge which influence the child's oral health.

IX. Clinical Variable

a) Early childhood caries

The results of this study demonstrated that 'd' component dominated the 'DMFT' score. The low level of dental treatment was attributed to limited accessibility to preventive and treatment services, unwillingness of practitioners to provide care for young children and primary teeth being a low priority for consideration of treatment because of a parental belief that they are temporary.

In the present study prevalence of ECC was found to be 28.8% which is similar to the study conducted by *Gopal et al*[14](27.3%)among preschoolers of Andhra Pradeshbut lower than the study conducted by *Singh S*[15] among 717 preschoolers (40%) of Bangalore city and *Sarvanan S*[16](44.4%) among 1009, 5 year old subjects of Pondicherry. This difference in the prevalence could be attributed to the fact that Faridabad has optimum or above optimum level of naturally present fluoride in the water which prevents caries. In the present study though there was found a higher prevalence of caries among females (29%) and 2-4 year (30.2%) subject group as compared to males (28.6%) and 5-6 year (26.4%) old subject group but this difference failed to reach the level of statistical significance. This finding is in contrast with the study conducted by *Infante & Gillespie et al*[17]*, Zerfowski et al*[18].

Relationship of ECC with oral hygiene status: Oral hygiene status of the preschoolers was assessed using Visible Plaque Index (VPI). In the present study poor oral hygiene was found among more than half of the study population (51.2%) and prevalence of ECC was significantly higher among subjects with visible plaque. These findings are in accordance with the study conducted by *Mohebbi et al*[19] *and Zhang et al*[20] where visible plaque was found in around 60 % of the study population significantly having more prevalence of ECC. This is due to the fact that the tooth surface loses some tooth mineral from the action of the acid formed by plaque bacteria leading to dental caries.

Relationship of ECC with parental attitude: In the present study prevalence of ECC was significantly less for those subjects who belonged to well educated families. Whereas *Qin et al*[21]concluded in their study that parental

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education level did not show any significant effect on the oral health of the children.

Relationship of ECC with parental frequency of cleaning: A study by *Vanagas et al*[22] has reported that oral hygiene skills and attitudes of parents toward children oral health are significantly associated with the development of oral hygiene skills including tooth brushing in their children.

Relationship of ECC with feeding Practices and ECC: In the present study, subjects who were not breastfed had significantly higher number of decayed surfaces. This finding is in corroboration with the studies conducted by *Eronat et al*[12] and *Yonezuet al.*[23] Mean number of decayed surfaces were found to be significantly higher among subjects who were breast fed for 6 months or 2 years than those who were breastfed for three years or more. This may be due to the reason that along with bottle feeding child was ad libitum breastfed as well.

Relationship of ECC with assistance provided to the child for brushing: Results of present study showed that mean number of decayed surfaces are significantly lower (0.35±0.69) in those children who perform toothbrushing under parents supervision. These findings are in agreement with the findings of the study conducted by *Hallett and O Rourkeet al*[24] who also reported that dental prevalence of dental caries were less in the subjects with supervised brushing.

Relationship of ECC with method of brushing: Higher mean number of decayed surfaces (0.70±1.43) was observed among those practicing horizontal method of brushing in comparison with other brushing directions; however, it was statistically significant ($p<0.05$). Young children can be advised for roll on technique keeping in view the efficiency of brushing. Similar findings were reported by *Wei and Hyman et al.*[25]

Relationship of ECC with frequency of brushing: Promoting tooth brushing in preschool children is of great relevance because this is a way to favor dental health by maintaining clean teeth. In the present study the caries prevalence was lower among those children brushing their teeth twice daily, the difference in the observations obtained was statistically significant ($p<0.05$). The findings of the present study were similar to the findings of *Bjarnason et al* [26], *Kuriakose and Joseph et al* [27] who also reported decreased prevalence of dental caries with increased frequency of brushing.

X. Limitations

One of the limitation of this study is that the questionnaire was given to the subject and was instructed to get it filled by their mother and father jointly. As the questionnaire was filled at home the investigator could not have any control over it. Probably, this could be the reason of high non response rate of many items. Thus further research with face to face interviews with the parents is recommended to eliminate the non-response bias.

XI. Conclusion

Parent's understanding was good related to many factors affecting oral health, but there still exist definite lacuna, which need to be considered. As prevention is always better than cure, parent's knowledge can be one of the main key factors in preventing oral diseases and promoting the oral health of their children. There is a need to enhance dental health education activities, targeting parents of preschool age, so that preventive strategies start at an early age.

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Streptococcus Salivarius Subsp *Salivarius* BIO5- Toxicity Evaluation of a Possible Probiotic Strain

By Fantinato V, Camargo HR & Sousa ALOPilleggi

Abstract- Probiotic bacteria, which are traditionally studied to aid intestinal functions, are also currently being researched for benefits in other parts of the body such as the oral cavity. In the case of throat infections caused by bacteria, which traditional treatment involves antibiotics, some researchers are studying the possibility of introducing special strains of commensal bacteria into the mouth that can specifically inhibit the tonsillitis agent *Streptococcus pyogenes* for both prevention and treatment of such disease. A PCR assay was used to identify *Streptococcus salivarius* subsp *salivarius*. Therefore, tests for the complete absence of toxicity of these strains are required to ensure their safe use in human trials. In this research, we evaluated the new *Streptococcus salivarius* subsp *salivarius* BIO5 strain for toxicity factors, as follows: clinical, ophthalmic lesions, behavioral observations, mortality, anatomopathological examination, biopsy, hematology, serum biochemistry, and urine analysis. This strain, isolated from the oral cavity and with bacteriocinogenic activity against S. pyogenes, showed a complete absence of toxicity and thus it may be used in replacement therapy as a probiotic.

Keywords: *Streptococcus salivarius, probiotics, bacteriocin, Streptococcus pyogenes, toxicity.*

GJMR-J Classification: *NLMC Code: WC 210*

STREPTOCOCCUSSALIVARIUSSUBSPSALIVARIUSBIO5TOXICITYEVALUATIONOFAPOSSIBLEPROBIOTICSTRAIN

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Streptococcus Salivarius Subsp *Salivarius* BIO5-Toxicity Evaluation of a Possible Probiotic Strain

Fantinato V [α], Camargo HR [σ] & Sousa ALOPilleggi [ρ]

Abstract- Probiotic bacteria, which are traditionally studied to aid intestinal functions, are also currently being researched for benefits in other parts of the body such as the oral cavity. In the case of throat infections caused by bacteria, which traditional treatment involves antibiotics, some researchers are studying the possibility of introducing special strains of commensal bacteria into the mouth that can specifically inhibit the tonsillitis agent *Streptococcus pyogenes* for both prevention and treatment of such disease. A PCR assay was used to identify *Streptococcus salivarius* subsp *salivarius*. Therefore, tests for the complete absence of toxicity of these strains are required to ensure their safe use in human trials. In this research, we evaluated the new *Streptococcus salivarius* subsp *salivarius* BIO5 strain for toxicity factors, as follows: clinical, ophthalmic lesions, behavioral observations, mortality, anatomopathological examination, biopsy, hematology, serum biochemistry, and urine analysis. This strain, isolated from the oral cavity and with bacteriocinogenic activity against *S. pyogenes*, showed a complete absence of toxicity and thus it may be used in replacement therapy as a probiotic.

Keywords: *Streptococcus salivarius, probiotics, bacteriocin, Streptococcus pyogenes, toxicity.*

I. Introduction

Probiotic bacteria, which are traditionally studied to aid intestinal functions, are also currently being researched for benefits in other parts of the body such as the oral cavity. Thus, commensal microorganisms of the mouth, ears, and nose have been researched for the prevention or treatment of oropharyngeal diseases such as periodontitis, caries, sore throat, and otitis.

This study aimed to evaluate the presence or absence of toxicity of the *Streptococcus salivarius* subsp *salivarius* BIO5 strain[1].

The *Streptococcus salivarius* subsp *salivarius* species is an indigenous microorganism of the mouth, and its niches are the tongue and mucous membranes. It is a bacterium that sets in soon after birth, remaining dominant from childhood through life[2, 3]. Some strains are capable of producing bacteriocin-like inhibitory substances (BLIS)[4] in vitro against the agent of bacterial tonsillitis - *Streptococcus pyogenes* or Group A Streptococcus (GAS). Tonsillitis is one of the most common childhood diseases, which when repeated may lead to the development of rheumatic fever and cause severe sequels[5]. Considering this knowledge, the possibility of performing replacement therapy was considered by introducing positive bacteriocin strains through some vehicle into the oral cavity of children with tonsillitis. The purpose of performing toxicity tests with an indigenous microorganism of the oral cavity is that this species - *Streptococcus salivarius* subsp salivarius- has been studied as the ideal probiotic for controlling bacterial tonsillitis caused by *Streptococcus pyogenes*. Despite being a beneficial and non-pathogenic saprophytic microorganism, it is necessary to confirm that the strain in question is completely safe. As a probiotic, *S. salivarius* has been used with the K12 strain successfully through oral tablets preceded by a mouthwash with an antiseptic[6, 7, 8].

The BIO5 strain of *Streptococcus salivarius* has been studied for the development of a product other than the K12 strain that is administered through tablets. Lately, new strains of *S. salivarius* have been studied for the same purpose: control and treatment of tonsillitis as well as otitis and halitosis[9, 10].

Therefore, confirmatory tests for the complete absence of toxicity of the *Streptococcus salivarius* BIO5 strain are required.

II. Material & Methods

We obtained one strain with clear evidence of bacteriocin-like production and inhibition against most of the 100 strains of *S. pyogenes*. The *Streptococcus salivarius* subsp *salivarius* BIO5 strain was tested by PCR11 to identify *S. salivarius*.PCR primers specific for *S. salivarius*, Ssa442F (5´-AAC GTT GAC CTT ACG CTA GC-3´) and Ssa2712R (5´-GAT TCT GTC AAA GAA GCC AC-3´) were used to amplify a 2271 bp fragment from dextranase (dex) gene. Isolates were prepared for PCR by pelleting 3 ml of bacteria grown in BHI broth, subsequently suspended in 1 ml of sterile MilliQ water. 5 μl aliquots of the cell suspension were used in a 50 μl reaction containing Reaction Buffer Biotools 1x with 2 mM MgCl2 (Madrid, Spain), 1 μM of each primer (Invitrogen, Carlsbad, CA, USA), 2 mMdNTPs mix (Invitrogen) and 2, 0 U of Taq polymerase (Invitrogen). The amplification reaction was performed in an Eppendorf Mastercycler gradient thermal cycler (Eppendorf, Hamburg, Germany) as described bylgarashi et al11 as it follows: 95°C for 10 min followed

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by 26 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 1 min. The last cycle comprised denaturation at 94°C for 1 min, annealing at 55°C for 1 min and final extension step at 72°C for 5 min. The PCR fragments were subjected to electrophoresis on 1.5% agarose gel in TAE buffer 1x and stained with ethidium bromide.

Toxicity tests

The toxicity tests for this study were clinical, ophthalmic lesions, behavioral observations, mortality, anatomopathological examination, biopsy, hematology, serum biochemistry, and urine analysis[12]. The authors complied with the guidelines for the care and use of laboratory animals as described by the U.S. National Institutes of Health.

a) Test Product Preparation

The BIO5 strain was seeded from blood agar stock on Mitis Salivarius agar and incubated at 37°C/24h for confirmation of culture purity. From this growth, suspensions were prepared in saline solution at concentrations from 10^8 to 10^9 CFU/ml. This preparation was called Test Product.

b) Animal Studies

Animals of the Rattusnovergicus species, Wistar Lineage, albino rats were used. A total of 100 animals were initially used - 50 males and 50 females. These animals received three different doses of the Test Product for a period of 28 consecutive days, namely 1.0, 2.0, and 4.0 ml/kg. The animals originated from the breeding room of the company CIALLYX Laboratórios & Consultorias-Paulínia, São Paulo, Brazil.

- Administration in repeated doses, in a rodent (rat albinus "Wistar"), of the test product (1.0, 2.0 and 4.0 ml) by kg of body weight of 10 animals per dose (total of 50 males and 50 females), which received each of the dosages of the test product plus a control group and a "recovery group".
- Clinical, visual, and behavioral observations were made over the entire period of the treatment of the animals with different doses of the test product, i.e., for 28 consecutive days. There were two daily observations: in the morning and the afternoon.
- Samples of blood/plasma (with the help of a syringe moistened with heparin or not) were collected for additional tests including complete hematology, serum biochemistry tests, electrolytes, and urine analysis.

III. Results

a) Experimental Design

GROUP	MALE	FEMALE
Controlgroup	10	10
Dose 1,0ml/kg	10	10
Dose 2,0ml/kg	10	10
Dose 4,0ml/kg	10	10
Dose 4,0ml/kg (Product test "Recovery")	10	10
Animal & Total	50	50

- Mortality

No deaths were detected throughout the acclimation phase and experimentation (Table 1).

- Anatomopathological examination

All animals that survived the various treatments after 28 days of observation were deeply anesthetized and euthanized ethically. The procedure was necropsy on all rats. Potential injuries were recorded and tissue was collected for histopathological examinations.

- Biopsy

No lesions were found in organic and/or any substantial tissue of animals examined by necropsy (adrenal, aorta, articulation, spleen, bladder, brain, cerebellum and pons, heart, esophagus, stomach, pylorus and cardio, epididymis, salivary gland, liver, testicle, duodenum, ileum, jejunum, cecum, colon, rectum, larynx, lymph nodes, bone marrow, skeletal muscle, prostate, thyroid, pancreas, skin, lungs, kidneys, thymus, thyroid, and trachea.

- Test substance administration

No problems related to the Test Product administration were found.– Changing the standard of feed and water consumption No changes were observed in the patterns of water and feed intake, both in the acclimation phase (day zero to day seven) and throughout the experimental period (from day zero to day 28).

- Daily observations

No changes were detected with behavioral and/or clinical significance during the entire acclimation phase and experimentation.

- Ophthalmology

There were no problems and/or specific ophthalmic lesions in any of the animals treated

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throughout the acclimation phase and/or experimentation.

- Hematology

Different hematological parameters were analyzed in animals treated with repeated doses of the test product observed for 53 consecutive days, and no changes were verified (Table 2).

- Serum biochemistry

Different parameters of serum biochemistry were analyzed in animals treated with repeated doses of the test product observed for 53 consecutive days, and no changes were verified (Table 3).

- Urine analysis

Different parameters of urine were analyzed in animals treated with repeated doses of the test product observed for 53 consecutive days (Table 4). Urine analysis (quantitative method) of 10 animals (males) per group receiving doses of 1.0, 2.0, and 4.0 ml/kg. Averages or rankings for the following parameters: color, turbidity, pH, presence of leukocytes (cells/ml),presence of glucose (mg/dL), bilirubin, and presence of blood (erythrocytes/ml).

Table 1: Number of animals used in each group, number of dead animals and the percentage of deaths for the three treatment groups performed at a single dose

Group	Product Dose	Animal number	Sex	Number Death	Mortality %
1	Product Test / 1,0 ml/kg	10	M	0	0
2	Product Test / 2,0 ml/kg	10	M	0	0
3	Product Test / 4,0 ml/kg	10	M	0	0
4/"Recovery"	Product Test / 4,0 ml/kg	10	M	0	0
1	Product Test / 1,0 ml/kg	10	F	0	0
2	Product Test / 2,0 ml/kg	10	F	0	0
3	Product Test / 4,0 ml/kg	10	F	0	0
4/"Recovery"	Product Test / 4,0 ml/kg	10	F	0	0

Table 2: Hematological evaluation of ten animals (male) group, which received the dose of 1.0, or 2.0 or 4.0 ml / kg of Test Product

Male	Control	Low set Dose ml/kg	Intermed Dose ml/kg	Highest Dose ml/kg	Normal Values Rats[A]	Unit
Total number erythrocytes	7,6	7,2	7,7	8,1	6,0-9,0	10^{12}/L
Hemoglobinconcentration	14,1	14,7	14,9	15,4	11,0-17,0	g/dL
Hematocrites	44,3	42,4	41,5	42,0	38,0-50,0	%
MCHC	31,9	31,7	32,6	32,9	31,0-36,0	g/dL
% reticulocites	0	0	1	1	1,0-4,0	% RBC
Total number granulocytes	4,7	4,4	4,9	5,1	1,0-6,0	10^9/L
% de granulocytes	16,8	17,8	18,9	22,2	5,0-30,0	%
Numberlymph /monoc	7,5	7,3	6,6	5,9	4,0-16,0	10^9/L
Total leukocytes	12,8	12,2	11,6	13,7	4,0-17,0	10^9/L
Numberlymphocytes	8,3	9,6	9,2	9,2	3,0-15,0	10^9/L
Numberneutrophils	3,7	4,1	5,1	5,2	0,0-6,0	10^9/L
Numbermonocytes	1,0	1,4	1,1	1,5	0,2-2,0	10^9/L
Numbereosinophils	0,1	0,2	0,4	0,2	0,0-0,5	10^9/L
Numberbasophils	0	0	0	0	Raros	10^9/L
Numberplatelets	756	657	678	458	800-1400	10^9/L
Coagulation time	194	192	224	324	100-500	s

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Table 3: Biochemical tests of serum of ten animals (male) by the groups that received doses of 1.0, or 2.0 or 4.0 ml / kg of the test product – Males

	Carrier	Dose ml/kg	Dose ml/kg	Dose ml/kg	Values Rats[(A)]	Units
	ligth	ligth	ligth	ligth	ligth	
Color	yellow	yellow	yellow	yellow	yellow	
	transluce	transluce	transluce	transluce	transluce	
pH	7,5	7,1	7,7	7,1	6,0 - 8,0	
Leukocytes	25	25	0	0	until 100	Leucocytes/ ml
Protein	30	30	0	0	until 100	mg/dl
Glucose..	Negative	Negative	Negative	Negative	Negative	Negative
Ceton	0	15	0	15	until 15	mg/dl
Urobilinogen	Normal	Normal	Normal	Normal	Normal	mg/dl
Bilirrubin	Negative	Negative	Negative	Negative	Negative	
Blood.	Negative	Negative	Negative	Negative	Negative	erythrocytes /ml

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Table 4: Analysis of urine (quantitative method) of ten animals (male) group received the doses of 1.0, or 2.0 or 4.0 ml / kg

	Control	Lowest Dose ml/kg	Intermed Dose ml/kg	Highest Dose ml/kg	Normal Value rats (A)	Units
Total Protein	7,3	7,2	7,0	6,8	5,0-8,0	g/dL
Albumin	4,0	4,2	4,1	3,9	3,0-5,0	g/dL
Globulin	2,2	2,6	2,1	2,6	1,0-3,0	g/dL
Relationalbumim/ globulin	1,5	1,1	1,2	1,4	0,0-2,0	g/dL
Aspartateaminotransferase	76,8	65,8	72,4	74,4	20,0-100,0	IUL
Alaninaminotransferase	54,6	56,8	66,5	67,2	10,0-80,0	IU/L
Alkalinephosphatase	89,9	99,6	98,4	78,8	70,0-450,0	IU/L
Total bilirubin	0,3	0,2	0,2	0,3	0,0-0,5	mg/dL
Creatinin	0,4	0,5	0,5	0,7	0,1-0,8	mg/dL
Ureanitrogena	13,4	13,6	13,5	12,6	12,0-24,0	mg/dL
Glucose	123,5	143,6	133,7	142,9	140,0-220,0	mg/dL
Total Colesterol	57,8	67,8	77,8	65,7	20,0-110,0	mg/dL
Calcium	10,3	11,5	11,3	11,5	10,0-12,0	109/L
Phosphorus	6,0	5,9	6,1	6,6	5,0-7,0	mg/dL
Sodium	138,4	140,2	142,4	144,3	136,0-154,0	Mmol/L
Chloride	98,3	95,5	98,3	92,4	98,0-110,0	Mmol/L
Potassium	5,5	5,6	5,2	7,1	5,0-7,0	Mmol/L

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IV. Discussion

All articles published on the *Streptococcus salivarius* subsp *salivarius* species agree that this microorganism is an indigenous and saprophyte inhabitant of the human oral cavity. It is installed on the tongue and mucous membranes shortly after birth, remaining in these habitats for life [3, 4]. It is an abundant and predominant isolate of the human oral microbiota with the basic function of protecting this environment by hindering or preventing the entry of other foreign or pathogenic microorganisms, aiding the control of the ecological balance of the microbiota and oral health.

In recent years, several authors have been studied the *Streptococcus salivarius* K12 strain as a probiotic in the treatment of tonsillitis caused by *S. pyogenes*, showing promising results [6,7,8].

As the *S. salivarius* species has only recently been used as a probiotic, when searching the literature for toxicity data of this species we found rare reports of toxicity studies, because it is a saprophytic and indigenous microorganism of the oral cavity. The only study on *S. salivarius* toxicity was carried out with the K12 strain, in which the authors analyzed toxicity, antibiotic resistance, virulence determinants, the production of deleterious metabolic by-products, and genetic stability[13]. The various sets of data obtained in this study showed no evidence of toxicity and no acute or subacute toxicity effects associated with the K12 strain, leading to the cure of tonsillitis and otitis.

La Mantia et al[9] used the *S. salivarius* 24SMB and *Streptococcus oralis* 89a nasal spray Rinogermina for preventing recurrent acute otitis media in children. The results showed that all actively treated children with the highest acute otitis had a reduction of recurrence, whereas only 50% of the children in the control group had reduced the disease.

De Grandi et al[10] studied the efficiency of nasal instillation of *S. salivarius* 24SMBc and *S. oralis* 89a. In particular, nasal swabs were sampled one, two, and four weeks after seven days of treatment with Rinogermina. They analyzed modulations of the abundance of pathogenic species such *as Corynebacterium diphtheriae, Haemophilus parainfluenzae, Moraxella catarrhalis, Prevotella denticola, Prevotella melaninogenica, Rothia dentocariosa, Staphylococcus aureus,* and *Streptococcus pseudopneumoniae*. They characterized a significant temporary decrease in those species. The beneficial effects of *S. salivarius* 24SMBc and *S. oralis*89a nasal intake were assessed but seemed to be restricted in specific temporal windows.

Marquart et al[14] studied virulence aspects of 22 streptococci strains isolated from endophthalmitis, one of which was *Streptococcus salivarius*. This strain showed amikacin resistance, vancomycin sensitivity, and intermediate ceftazidime sensitivity. Bacterial genomic DNA from each of the 22 isolates was tested for the presence of the gene encoding pneumolysin, ply. This base pair gene is present in *S. pneumoniae* and encodes a cytotoxin involved in virulence, but the *S. salivarius* strain did not present it. The protease activity was also negative for this strain, as well as cytotoxicity or hemolytic.

To identify putative probiotics, Frick et al[15] tested some commensal bacteria on their toxicity, invasiveness, inhibition of Yersinia-induced inflammation in vitro and in vivo, and modulation of dextran sodium sulfate (DSS)-induced colitis in mice. None of the commensal bacteria tested, including *Streptococcus salivarius*, was toxic for or invaded the epithelial cells.

As the authors cited, despite different studies, our results also showed the absence of any virulent activity in vivo and in vitro, proving that the *S. salivarius* BIO5 strain can be used as a probiotic in the treatment or prevention of bacterial tonsillitis or other infections in humans.

The K12 strain is administered through tablets. Beforeits administration, a mouthwash with strong antiseptics is required to eliminate part of the microbiota and facilitate the introduction of the strain in question. The authors who used *S. salivarius* 24SMB strains used an intranasal administration [9, 10].

As we are discussing new bacteria and proposing their use as probiotics, it is appropriate to refer to the Food and Agriculture Organization (FAO), World Health Organization (WHO), and the International Scientific Association for Probiotics and Prebiotics (ISAPP).

In 2001, the Food and Agriculture Organization of the United Nations and the World Health Organization[16] debated the field of probiotics, starting with the definition: “live microorganisms, which when administered in adequate amounts confer a health benefit on the host”.

Revisiting the term ‘probiotic’ in October 2013 by the International Scientific Association for Probiotics and Prebiotics (ISAPP)[17] to discuss the field of probiotics, a more grammatically correct definition would be worded as, “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host”. The differentiation between probiotic and commensal was also established. Although probiotics are isolated from normal microbiota, as long as they are not isolated and proven to be beneficial to health, they cannot be called probiotics.

As the literature already shows several works published successfully on the treatment of tonsillitis, especially in children, with *S. salivarius*, we aim to use the BIO5 strain in the treatment and prevention of this disease but using a different vehicle from those used so far.

Confirming the absence of toxicity of the *Streptococcus salivarius* subsp *salivarius* BIO5 strain, we

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feel confident in performing the next step, i.e. the study in humans, with this new strain, hoping we can call it a probiotic.

V. Conclusion

The test fulfilled the purposes to evidence potential toxic effects on animals - males and females - in any of the doses (1.0, 2.0, 4.0 ml/kg). Neither hematological nor pathological changes were observed in serum biochemistry among many different groups. The results obtained allowed us to affirm that the test product did not induce toxic effects evident in doses used of the Test Product analyzed, which is *Streptococcus salivarius* subsp *salivarius* BIO5. Therefore, the *Streptococcus salivarius* BIO5 strain can be safely used in the prevention or treatment of bacterial tonsillitis in humans as it has been shown that such strain did not cause any toxic effects in animals.

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For scanned images, the scanning resolution at final image size ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs): >350 dpi; figures containing both halftone and line images: >650 dpi.

Color charges: Authors are advised to pay the full cost for the reproduction of their color artwork. Hence, please note that if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a Color Work Agreement form before your paper can be published. Also, you can email your editor to remove the color fee after acceptance of the paper.

Tips for writing a good quality Medical Research Paper

1. Choosing the topic: In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

2. Think like evaluators: If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

3. Ask your guides: If you are having any difficulty with your research, then do not hesitate to share your difficulty with your guide (if you have one). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work, then ask your supervisor to help you with an alternative. He or she might also provide you with a list of essential readings.

4. Use of computer is recommended: As you are doing research in the field of medical research then this point is quite obvious. Use right software: Always use good quality software packages. If you are not capable of judging good software, then you can lose the quality of your paper unknowingly. There are various programs available to help you which you can get through the internet.

5. Use the internet for help: An excellent start for your paper is using Google. It is a wondrous search engine, where you can have your doubts resolved. You may also read some answers for the frequent question of how to write your research paper or find a model research paper. You can download books from the internet. If you have all the required books, place importance on reading, selecting, and analyzing the specified information. Then sketch out your research paper. Use big pictures: You may use encyclopedias like Wikipedia to get pictures with the best resolution. At Global Journals, you should strictly follow here.



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6. Bookmarks are useful: When you read any book or magazine, you generally use bookmarks, right? It is a good habit which helps to not lose your continuity. You should always use bookmarks while searching on the internet also, which will make your search easier.

7. Revise what you wrote: When you write anything, always read it, summarize it, and then finalize it.

8. Make every effort: Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

9. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several unnecessary diagrams will degrade the quality of your paper by creating a hodgepodge. So always try to include diagrams which were made by you to improve the readability of your paper. Use of direct quotes: When you do research relevant to literature, history, or current affairs, then use of quotes becomes essential, but if the study is relevant to science, use of quotes is not preferable.

10. Use proper verb tense: Use proper verb tenses in your paper. Use past tense to present those events that have happened. Use present tense to indicate events that are going on. Use future tense to indicate events that will happen in the future. Use of wrong tenses will confuse the evaluator. Avoid sentences that are incomplete.

11. Pick a good study spot: Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

12. Know what you know: Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

13. Use good grammar: Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

14. Arrangement of information: Each section of the main body should start with an opening sentence, and there should be a changeover at the end of the section. Give only valid and powerful arguments for your topic. You may also maintain your arguments with records.

15. Never start at the last minute: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

16. Multitasking in research is not good: Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

17. Never copy others' work: Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

18. Go to seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

19. Refresh your mind after intervals: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.

20. Think technically: Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

21. Adding unnecessary information: Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn't be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

22. Report concluded results: Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

23. Upon conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

Final points:

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

The introduction: This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

The discussion section:

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear: Adhere to recommended page limits.



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Mistakes to avoid:

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

Title page:

Choose a revealing title. It should be short and include the name(s) and address(es) of all authors. It should not have acronyms or abbreviations or exceed two printed lines.

Abstract: This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

Introduction:

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.

The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

Approach:

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

Procedures (methods and materials):

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

Materials may be reported in part of a section or else they may be recognized along with your measures.

Methods:

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

Approach:

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

What to keep away from:

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.



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Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

What to stay away from:

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:

As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

Figures and tables:

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

Discussion:

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an argument should be.

Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."

Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

The Administration Rules

Administration Rules to Be Strictly Followed before Submitting Your Research Paper to Global Journals Inc.

Please read the following rules and regulations carefully before submitting your research paper to Global Journals Inc. to avoid rejection.

Segment draft and final research paper: You have to strictly follow the template of a research paper, failing which your paper may get rejected. You are expected to write each part of the paper wholly on your own. The peer reviewers need to identify your own perspective of the concepts in your own terms. Please do not extract straight from any other source, and do not rephrase someone else's analysis. Do not allow anyone else to proofread your manuscript.

Written material: You may discuss this with your guides and key sources. Do not copy anyone else's paper, even if this is only imitation, otherwise it will be rejected on the grounds of plagiarism, which is illegal. Various methods to avoid plagiarism are strictly applied by us to every paper, and, if found guilty, you may be blacklisted, which could affect your career adversely. To guard yourself and others from possible illegal use, please do not permit anyone to use or even read your paper and file.

Criterion for Grading a Research Paper (Compilation) by Global Journals

Please note that following table is only a Grading of "Paper Compilation" and not on "Performed/Stated Research" whose grading solely depends on Individual Assigned Peer Reviewer and Editorial Board Member. These can be available only on request and after decision of Paper. This report will be the property of Global Journals.

Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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