tania saskianti <tania-s@fkg.unair.ac.id>

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1 message

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Reply-To: International Journal of Developmental Disabilities <yjdd-peerreview@journals.tandf.co.uk>
To: Tania Saskianti <tania-s@fkg.unair.ac.id>

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International Journal of Developmental Disabilities

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Invitation to review JDD1087

1 message

International Journal of Developmental Disabilities <em@editorialmanager.com> Mon, Feb 14, 2022 at 9:43 AM
Reply-To: International Journal of Developmental Disabilities <yjdd-peerreview@journals.tandf.co.uk>
To: Tania Saskianti <tania-s@fkg.unair.ac.id>

JDD1087
EVALUATION OF SALIVARY BIOMARKER LEVELS IN CHILDREN WITH DOWN SYNDROME WITH DENTAL CARIES
International Journal of Developmental Disabilities

Dear Tania Saskianti

We have received the above submission to International Journal of Developmental Disabilities and should be grateful if you could very kindly agree to review it and comment on its technical merit and suitability for publication.

The International Journal of Developmental Disabilities(IJDD) which was formerly the British Journal of Developmental Disabilities has a history of more than fifty years of publishing quality research, review and points of view articles on all practical aspects of intellectual disabilities.

The abstract of the submission is as follows:
Purpose- To assess the salivary pH, buffer capacity, viscosity, and Malondialdehyde (MDA) in children with Down syndrome with dental caries, before and after treatment. Methodology- A total of 15 children with Down syndrome between the age groups of 6-14 years, who reported to the Nitte Special Child Care Centre (N-SPECC), Department of Pediatric and Preventive Dentistry, Department of Pediatric and Preventive Dentistry, A B Shetty Memorial Institute of Dental Sciences were selected for the study. Salivary samples were collected to get the baseline values for evaluating the salivary MDA, pH, viscosity, and buffering capacity. The levels of MDA were estimated using TCA-TBA-HCl reagent and the optical density of pink color formed was directly proportional to the concentration of MDA in the given samples. Salivary pH and buffering capacity were estimated using Saliva check buffer kit and viscosity was measured using Ostwald viscometer. The patients were recalled after a period of 2 weeks and oral health education and saliva samples were collected and evaluated for the above parameters post-treatment. Results were subjected to statistical analysis. Results- There was a statistically significant difference seen for the values between the time intervals (2 weeks) ($p<0.01$) for salivary MDA, viscosity, and buffering capacity levels in children with Down syndrome with dental caries post-treatment. Conclusion- The levels of salivary MDA and viscosity decreased significantly and furthermore, the levels of buffering capacity increased significantly post-treatment in children with Down syndrome. Evaluation of these biomarkers can be good adjuncts in caries risk assessment in these children.

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We should appreciate receiving your review within 26 days. Please submit your comments online at the above URL. There you will find guidance on the procedure for submitting confidential comments to the editor, comments for the author, completing the report form and uploading track changes. On accepting this invitation you will also receive a detailed letter to your email account guiding you through the review process.

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Brian Salmons, PhD
Editor
International Journal of Developmental Disabilities (formerly the British Journal of Developmental Disabilities)

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Review of JDD1087 due soon

1 message

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To: Tania Saskianti <tania-s@fkg.unair.ac.id>

Ref.: JDD1087
EVALUATION OF SALIVARY BIOMARKER LEVELS IN CHILDREN WITH DOWN SYNDROME WITH DENTAL CARIES
International Journal of Developmental Disabilities

Dear Tania Saskianti

We are making contact to remind you that your review of the above submission is due by 11 Mar 2022.

We would be grateful if you would submit your review as soon as possible at https://www.editorialmanager.com/JDD/.

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To download the paper now, please click the link:
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Kind regards,

Editor International Jnl of Dev Disabilities
Editorial Administrator
International Journal of Developmental Disabilities

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tania saskianti <tania-s@fkg.unair.ac.id>

Review of JDD1087

1 message

International Journal of Developmental Disabilities <em@editorialmanager.com> Wed, Mar 2, 2022 at 8:42 AM
Reply-To: International Journal of Developmental Disabilities <yjdd-peerreview@journals.tandf.co.uk>
To: Tania Saskianti <tania-s@fkg.unair.ac.id>

Ref.: JDD1087
EVALUATION OF SALIVARY BIOMARKER LEVELS IN CHILDREN WITH DOWN SYNDROME WITH DENTAL CARIES
International Journal of Developmental Disabilities

Dear Tania Saskianti

Thank you for your helpful comments on the above manuscript. We are most grateful for your assistance.

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Editor
International Journal of Developmental Disabilities

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tania saskianti <tania-s@fkg.unair.ac.id>

A decision has been made on JDD1087

1 message

International Journal of Developmental Disabilities <em@editorialmanager.com> Tue, Mar 29, 2022 at 1:58 PM
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To: Tania Saskianti <tania-s@fkg.unair.ac.id>

29 Mar 2022

Ref.: Ms. No. JDD1087
EVALUATION OF SALIVARY BIOMARKER LEVELS IN CHILDREN WITH DOWN SYNDROME WITH DENTAL CARIES
International Journal of Developmental Disabilities

Dear Tania Saskianti,

A decision of Return for Mandatory Revisions has been made on EVALUATION OF SALIVARY BIOMARKER LEVELS IN CHILDREN WITH DOWN SYNDROME WITH DENTAL CARIES, the manuscript that you recently reviewed for International Journal of Developmental Disabilities.

Thank you for taking the time to review this manuscript.

Best Regards,

Narasimhan Vaidyanathan
Support Administrator
International Journal of Developmental Disabilities
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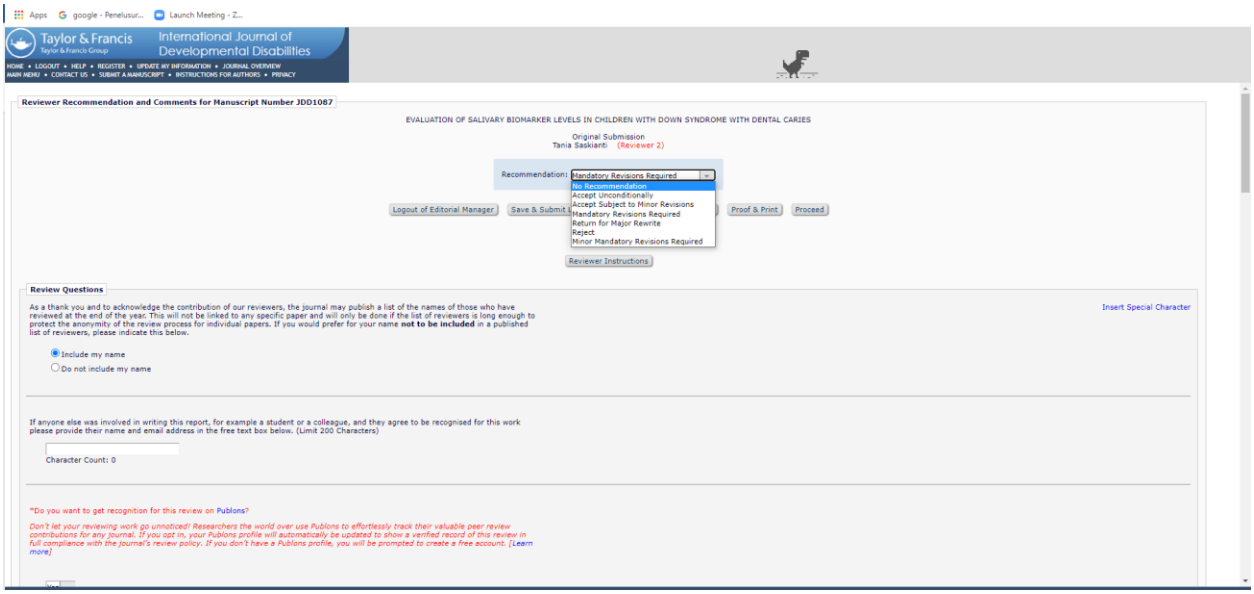

*Is the paper better suited as a Points of View article? (i.e. either it is a case study/reports nonstatistically relevent findings or it is provocative rather than factual)

Yes

*Is the standard of English acceptable? Note that authors are now being offered an English language polishing service; thus papers should be rejected if the English is of poor quality.

Yes

*Is the preferred terminology used consistently throughout the paper? Acceptable terminology includes: people with intellectual disabilities, persons with intellectual disabilities, children with intellectual disabilities or adults with intellectual disabilities.

Yes

*Is the paper clearly written for a diverse readership?

Yes

*Is the list of references relevant, appropriate and comprehensive? [Instructions]

No

Please enter any comments below (Limit 500 Characters)

- Several references were >10years
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International Journal of Developmental Disabilities

EVALUATION OF SALIVARY BIOMARKER LEVELS IN CHILDREN WITH DOWN SYNDROME WITH DENTAL CARIES

--Manuscript Draft--

Manuscript Number:	JDD1087
Full Title:	EVALUATION OF SALIVARY BIOMARKER LEVELS IN CHILDREN WITH DOWN SYNDROME WITH DENTAL CARIES
Article Type:	Original Research Paper
Keywords:	salivary biomarker; dental caries; lipid peroxidation; Down syndrome
Abstract:	Purpose- To assess the salivary pH, buffer capacity, viscosity, and Malondialdehyde (MDA) in children with Down syndrome with dental caries, before and after treatment. Methodology- A total of 15 children with Down syndrome between the age groups of 6-14 years, who reported to the Nitte Special Child Care Centre (N-SPECC), Department of Pediatric and Preventive Dentistry, Department of Pediatric and Preventive Dentistry, A B Shetty Memorial Institute of Dental Sciences were selected for the study. Salivary samples were collected to get the baseline values for evaluating the salivary MDA, pH, viscosity, and buffering capacity. The levels of MDA were estimated using TCA-TBA-HCl reagent and the optical density of pink color formed was directly proportional to the concentration of MDA in the given samples. Salivary pH and buffering capacity were estimated using Saliva check buffer kit and viscosity was measured using Ostwald viscometer. The patients were recalled after a period of 2 weeks and oral health education and saliva samples were collected and evaluated for the above parameters post-treatment. Results were subjected to statistical analysis. Results- There was a statistically significant difference seen for the values between the time intervals (2 weeks) ($p<0.01$) for salivary MDA, viscosity, and buffering capacity levels in children with Down syndrome with dental caries post-treatment. Conclusion- The levels of salivary MDA and viscosity decreased significantly and furthermore, the levels of buffering capacity increased significantly post-treatment in children with Down syndrome. Evaluation of these biomarkers can be good adjuncts in caries risk assessment in these children.

Figure 1: Dental saliva pH indicator

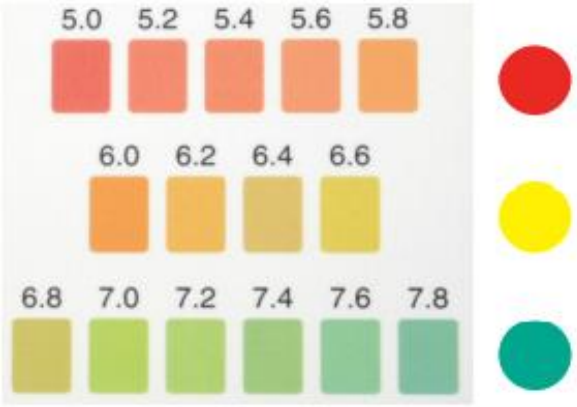

Figure 2: Saliva Check Buffer Testing Mat

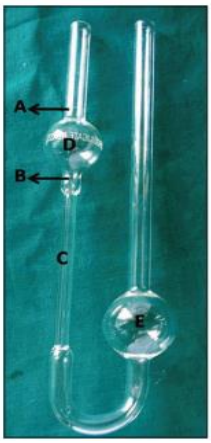

Figure 3: Ostwalds viscometer

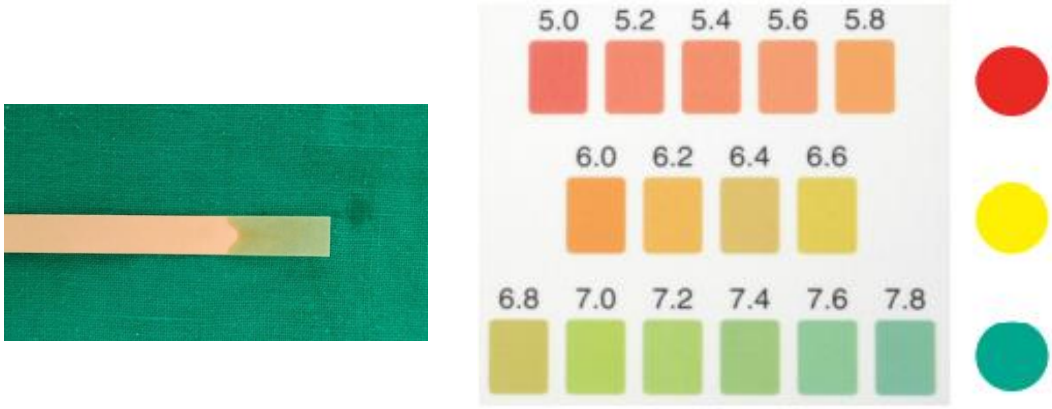

Figure 4: Estimation of salivary pH

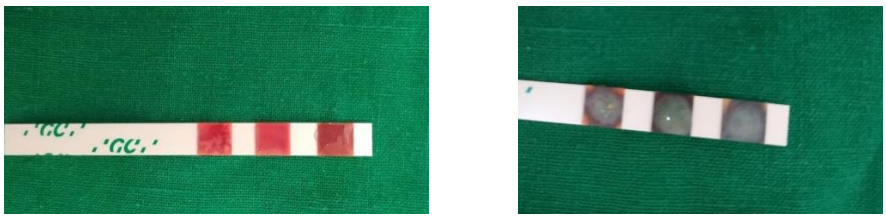

Figure 5: Estimation of buffering capacity of saliva

Table 1: Salivary levels of MDA (uM) pre and post intervention in children with Down syndrome

	Mean	Std. Deviation	Median	Z value	p value of Wilcoxon Signed Ranks Test
Pre intervention MDA values (uM)	.692000	.1453665	0.74	-3.415	0.001**
Post intervention MDA values (uM)	.253333	.1515476	0.22		

Table 2: Salivary pH pre and post treatment in children with Down syndrome

	Mean	Std. Deviation	Median	Z value	p value of Wilcoxon Signed Ranks Test
SALIVARY pH Pre-treatment	6.580	.1740	6.6	-1.630	0.102#
SALIVARY pH Post-treatment	6.507	.1280	6.4		

Table 3: Salivary viscosity (cP) pre and post treatment in children with Down syndrome

	Mean	Std. Deviation	Median	Z value	p value of Wilcoxon Signed Ranks Test
Pre-treatment Salivary Viscosity	1.077333	.0550930	1.05	-3.070	0.002**
Post-treatment Salivary Viscosity	1.0387	.02264	1.04		

Table 4: Salivary buffering capacity pre and post treatment in children with Down syndrome

	Mean	Std. Deviation	Median	Z value	p value of Wilcoxon Signed Ranks Test
Pre- treatment BUFFERING CAPACITY	4.87	1.685	5	-3.220	0.001**
Post-treatment BUFFERING CAPACITY	6.67	1.447	7		

Title: EVALUATION OF SALIVARY BIOMARKER LEVELS IN CHILDREN WITH DOWN SYNDROME WITH DENTAL CARIES

Abstract

Purpose- To assess the salivary pH, buffer capacity, viscosity and Malondialdehyde (MDA) in children with Down syndrome with dental caries, before and after treatment. **Methodology**- A total of 15 children with Down syndrome between the age groups of 6-14 years, who reported to the Nitte Special Child Care Centre (N-SPECC), Department of Pediatric and Preventive Dentistry, Department of Pediatric and Preventive Dentistry, A B Shetty Memorial Institute of Dental Sciences were selected for the study. Salivary samples were collected to get the baseline values for evaluating the salivary MDA, pH, viscosity and buffering capacity. The levels of MDA were estimated using TCA-TBA-HCl reagent and optical density of pink color formed was directly proportional to concentration of MDA in the given samples. Salivary pH and buffering capacity were estimated using Saliva check buffer kit and viscosity was measured using Ostwald viscometer. The patients were recalled after a period of 2 weeks and oral health education and saliva samples were collected and evaluated for above parameters post treatment. Results were subjected to statistical analysis. **Results**- There was a statistically significant difference seen for the values between the time intervals (2 weeks) ($p<0.01$) for salivary MDA, viscosity and buffering capacity levels in children with Down syndrome with dental caries post treatment. **Conclusion**- The levels of salivary MDA and viscosity decreased significantly and furthermore, the levels of buffering capacity increased significantly post treatment in children with Down syndrome. Evaluation of these biomarkers can be good adjuncts in caries risk assessment in these children.

Keywords: salivary biomarker, dental caries, lipid peroxidation, Down syndrome

MAIN TEXT

Introduction:

Down syndrome is the most common genetic birth disorder occurs, also known as trisomy 21. Children with down syndrome are vulnerable to multiple diseases such as hematologic disorders, cardiovascular alterations and immunological impairment in addition to cognitive defects. They are also susceptible to age related neural degenerative diseases. Excessive oxidative stress (OS) is thought to play a critical role in accelerated cell aging and neurologic disorders that are often seen in individuals with Down Syndrome and can result from defects in reactive oxygen species (ROS) metabolism. Antioxidant defence involves both enzymatic and non-enzymatic pathway which protect against oxygen radical damage. Superoxide dismutase (SOD-1) is an antioxidant enzyme encoded on 21 chromosome (21q22.1) which causes dismutation of superoxide anion into hydrogen peroxide and oxygen. The activity of SOD-1 is elevated in Down syndrome due to gene dosage effect. This elevated activity of SOD-1 induces oxidative stress by elevating reactive oxygen species (Sulthana M *et al.* 2012) Hence, the elevated levels of products of oxidative damage (Malondialdehyde) reflects significant oxidative stress in Down syndrome. Preventive dental care is one of the most common, of the unmet needs of these children, due to high prevalence of dental caries and need for restorative procedures. Marker of oxidative stress [Lipid peroxidation product Malondialdehyde (MDA)] is found in saliva and forms an important etiologic factor in dental caries (Murray RK *et al*. 2009)

Dental caries is a multifactorial inflammatory disease because bacterial toxins activate the matrix metalloproteinase such as collagenase, which causes breakdown of collagen matrix in the dentin and initiates the process of dental caries (Dean JA 2015) Any inflammatory

process initiates lipid peroxidation reaction that leads to the production of MDA which in turn alters the immunological mediators such as salivary peroxidase system. It affects the immune mechanism in saliva, leading to initiation and progression of bacterial infections such as dental caries (Dean JA 2015)

Lipid peroxidation cause alterations in the structure and function of the host cells by producing malondialdehyde (MDA) as the by-product through following mechanism. The hydroxyl radical which is formed initiates a chain reaction which leads to lipid breakdown through peroxidation and breakdown of cell membrane. These lipid radicals can diffuse through membranes, thus modifying the structure and function of the membrane resulting in a loss of cell homeostasis (Tóthová L *et al.* 2015)

Saliva has a role in controlling the pathogenesis of plaque formation leading to reduced susceptibility of dental caries by production of certain chemical reactions (Dean JA 2015). Saliva acts as a first line of defence, the most important caries protective functions of saliva are the flushing and neutralizing effects which are dependent on the flow rate (FR) and buffering capacity (BC) and pH of saliva. Buffering capacity of saliva is based on the phosphate system, the carbonic acid, and bicarbonate system and gets reduced in caries active children (Southward K 2011). Low salivary pH promotes the growth of aciduric bacteria which then allows the acidogenic bacteria to proliferate creating an inhospitable environment for the protective oral bacteria. This allows for a shift in the oral environmental balance to favour cariogenic bacteria, which further lowers the salivary pH and the cycle continues (Klaunig J E *et al.*1998). Hence, a decrease in pH will result in demineralization of the tooth surface resulting in dental caries. Glycoproteins particularly mucins are responsible for the viscoelastic character of saliva giving a lubricating film that enables free movement of oral tissues. Elevated salivary viscosity has been found to be associated with an increased

occurrence of dental caries (Sruthi KS *et al.*2019)

Since there is a lack of mechanistic studies on the role of ROS or oxidative damage in relation to dental caries for children with Down syndrome, hence this present study is being conducted.

AIM:

To assess the salivary pH, buffer capacity, viscosity and Malondialdehyde (MDA) in children with Down syndrome with dental caries, before and after treatment.

OBJECTIVES:

To assess the following salivary parameters in children with Down syndrome

1. Buffer capacity

2. Viscosity

3. pH

4. MDA

To compare the inter-group and intra-group values and statistically analyze the results

MATERIALS AND METHODS

A total of 15 children with Down syndrome between the age groups of 6-14years of both genders, reporting to the Nitte Special child care centre (N-SPECC), Department of Pediatric and Preventive Dentistry, A.B. Shetty Memorial Institute of Dental Sciences were included for the study. An informed consent in English and the local language was obtained from the parents prior to the study. Ethical clearance from the institutional ethics board was obtained. (Cert no. ABSM/EC 57/2019)

PARTICIPANTS

Children with 3 or more active carious lesions. (ICDAS-II Code 3-6) (Jablonski-Momeni A *et al.* 2008) and those who are willing to participate in the study with parental consent were included.

EXCLUSION CRITERIA:

Children with arrested carious lesions and those who are severely ill and cannot be examined were excluded.

METHODOLOGY

A total of 15 children with Down syndrome between the age groups of 6-14years of both genders, who reported to the Nitte Special child care centre (N-SPECC), Department of Pediatric and Preventive Dentistry, A.B. Shetty Memorial Institute of Dental Sciences were selected for the study.

A thorough dental examination was performed on a dental chair using a mouth mirror,

blunt explorer and active carious lesions were identified using International caries detection and assessment system-II (ICDAS-II Code3-6) (Jablonski-Momeni A *et al.* 2008), and the oral findings were recorded for each patient and a profile was maintained for ease of follow up till completion of the study.

Salivary samples were collected from both the groups to get the baseline values for evaluating the salivary MDA, pH, viscosity, and buffer capacity before caries control. Treatment of dental caries was accomplished using following interventions:

Active carious lesions which showed localized enamel breakdown and underlying dark shadow from dentine, identified by ICDAS-II Code 3-5 (Jablonski-Momeni A *et al.* 2008), were excavated such that nidus of inflammation was removed, and they were restored with GC Fuji Gold Label Type IX Glass Ionomer Cement.

For carious lesions with extensive distinct cavity in dentine and involving pulp, identified by ICDAS-II Code 6 (Jablonski-Momeni A *et al.* 2008), pulp therapy was performed for them followed by which interim restorations were given using GC Fuji Gold Label Type IX Glass Ionomer Cement (Saber AM *et al*. 2019). Oral health education and motivation was provided by giving oral hygiene instructions and diet counselling was done. The patients in both the groups were recalled after a period of 2 weeks post caries control and saliva samples were collected.

Collection of sample*:*

Pre-operative saliva samples were collected from both the groups. All samples of unstimulated saliva were collected within a 10 min period between 9 am and 11 am to minimize any possible effect of diurnal variation. The unstimulated saliva was collected by requesting the child to sit in a quite environment in the “coachman position” and expectorate

for 5 minutes in to sterile pre-weighed graduated cylinders (Klaunig J E et al.1998). Samples were immediately transported to Central Research Laboratory KSHEMA to be centrifuged at 4000rpm for 15min at 4ºC.The supernatants will be stored at -80ºC. All collected samples of saliva were evaluated for pH, buffering capacity, viscosity and malondialdehyde (MDA).

Saliva check buffer kit for estimation of salivary pH, buffering capacity:

The "Saliva Check Buffer Testing Mat" (GC Dental Products Corp., Kasugai City, Aichi, Japan) was used to estimate the pH and buffering capacity of saliva.

Estimation of salivary pH using "Saliva Check Buffer Testing Mat":

The enclosed pH strip was taken and one end of it was placed into the sample of collected saliva sample for 10 seconds and then check the color of the strip. The color of the test strip was compared while the paper was still moist. The pH reading was noted and results were recorded. The results were determined by dental saliva pH indicator as follows:

Highly acidic saliva will be in the red section, pH 5.0 - 5.8.

Moderately acidic saliva will be found in the yellow section, pH 6.0 - 6.6.

Healthy saliva will be in the green section pH 6.8 - 7.

Estimation of salivary buffer capacity using "Saliva Check Buffer Testing Mat":

The buffer test foil pack was opened and the pipette was used to draw up some saliva from the collection cup. One drop of saliva was dispensed onto each of the 3 test pads. The test strip was turned on its side to drain excess saliva onto a tissue. After 2 minutes, the color of each pad was compared with the table below and the 3 scores were added to record the

results.

Ostwald viscometer for estimation of salivary viscosity:

Ostwald viscometer was used for estimation of salivary viscosity. The relative viscosity of saliva with respect to water was measured using the "Ostwald's Viscometer

Ostwald viscometer **[Figure 1]**, which is a simple device and accurate for measuring the viscosity of the liquid. The viscosity of the liquid was determined by a comparison with a standard such as water:

$$\eta_1/\eta_2 = t_1d_1/t_2d_2$$

where η_1 is viscosity of liquid 1; η_2 is viscosity of liquid 2.
; t_1 flow time of liquid 1; t_2 flow time of liquid 2.;

d_1 density of liquid 1; d_2 density of liquid 2

Laboratory procedure for estimation of salivary levels of MDA:

250 µL of saliva was diluted to 500 µL with distilled water. To the diluted sample 1 mL of trichloroaceticacid-thiobarbituric acid-hydrochloric acid reagent (TCA-TBA-HCl) was added.The centrifuged samples were kept in boiling water bath for 15 minutes. The reaction mixture was cooled and centrifuged and the supernatant was taken. Optical density of the pink color formed was read at 535nm using spectrophotometer against butanol. The optical density of pink color formed is directly proportional to concentration of MDA in the given sample.

STATISTICAL ANALYSIS:

All data were entered into a computer by giving coding system, proofed for entry errors. Data obtained was compiled on a MS Office Excel Sheet (v 2019, Microsoft Redmond Campus, Redmond, Washington, United States). Data was subjected to statistical analysis using Statistical package for social sciences (SPSS v 26.0, IBM). Descriptive statistics like Mean & SD, median for numerical data has been depicted. Normality of numerical data was checked using Shapiro-Wilk test & was found that the data for MDA values only followed a normal curve; hence parametric tests have been used for comparisons.

Intra group comparison was done using paired t test (upto 2 observations). Normality of numerical data was checked using Shapiro-Wilk test & was found that the data for all other variables did not follow a normal curve; hence non-parametric tests have been used for comparisons. Intra group comparison was done using Wilcoxon Signed rank test (upto 2 observations)

For all the statistical tests, $p<0.05$ was considered to be statistically significant, keeping α error at 5% and β error at 20%, thus giving a power to the study as 80%.

* = statistically significant difference ($p<0.05$)

** = statistically highly significant difference ($p<0.01$)

= non significant difference ($p>0.05$) … for all tables

RESULTS:

A total of 15 children with Down syndrome each, between the age groups of 6-14years of both genders, who reported to the Nitte Special child care centre (N-SPECC), Department of Pediatric and Preventive Dentistry, A.B. Shetty Memorial Institute of Dental Sciences were screened for active carious lesions identified using International caries detection and assessment system-II (ICDAS-II Code3-6) (Jablonski-Momeni A *et al. 2008)*

There was a statistically highly significant difference seen for the values between the time intervals ($p<0.01$) for salivary MDA values with higher values at pre treatment groups than post using Wilcoxon Signed Ranks Test. (Table1). There was a statistically non significant difference seen for the values between the time intervals ($p>0.05$)) for salivary pH values with higher values at pre treatment groups than post using Wilcoxon Signed Ranks Test. **(**Table 2)

There was a statistically highly significant difference seen for the values between the time intervals ($p<0.01$) for salivary viscosity values with higher values at pre-treatment groups than post using Wilcoxon Signed Ranks Test. (Table 4)

There was a statistically highly significant difference seen for the values between the time intervals ($p<0.01$) for salivary buffering capacity values with higher values at pre treatment groups than post using Wilcoxon Signed Ranks Test. (Table 5)

DISCUSSION:

The salivary biomarkers for diagnostics of oxidative stress related diseases should

be stable, accumulated in detectable concentrations, reflect specific oxidation pathways, and correlate with disease severity (Radhi NJ *et al*. 2013). Since saliva acts as the first line of defense against free radicals (Amerongen and Veerman, 2002; Battino et al.,2002); the dysbalance between the production of free radicals and antioxidant status is called oxidative stress. Considering free radicals are highly reactive and have a short half-life, the products formed from the reaction of ROS/RNS with cellular macromolecules, which is mediated via iron catalysed Haber –Weiss reaction, these are used preferentially as biomarkers of oxidative damage (Murray RK *et al.* 2009)

Therefore, Lipid peroxidation products, oxidized proteins, and products of DNA oxidation and fragmentation—are used for the assessment of oxidative stress (Rai K et al.2012). MDA is synthesised from fatty acids having two or more methylene-interrupted double bonds via the lipid peroxidation process (Seethalakshmi C *et al.* 2016). Oxidative stress increases levels of lipid peroxidation which play a major role in pathogenesis of various inflammatory diseases (Sulthana M *et al*. 2012)

Apart from MDA, the physical properties of saliva such pH, viscosity and buffering capacity also act as salivary biomarkers to identify and detect various diseases. The defense system of saliva includes oral clearance, buffering action, inherent innate immunity and calcium and phosphate ions for remineralization. Hence, the interaction of protective and pathologic factors in saliva and plaque biofilm, as well as the balance between the cariogenic and non-cariogenic microbial populations that reside in saliva, decides the caries process. Also, among the other protective factors, salivary viscosity plays an important role. Fresh mixed human saliva is viscoelastic fluid (Sruthi K S *et al.* 2019)

In Down syndrome children mouth breathing is commonly seen because of smaller nasal passages and a large protruding tongue. This increases the viscoelasticity of saliva.

Glycoproteins particularly mucin are responsible for the viscoelastic character of saliva giving a lubricating film that enables free movement of oral tissues. Measuring salivary viscosity is of paramount importance since an elevated salivary viscosity is found to be associated with an increased occurrence of dental caries (Yarat A *et al*.1999). Buffering capacity is another physical property of saliva that serves an important biomarker for early detection of caries (Stabholz A et al.1991)

In the present study, there was a statistically high significant difference seen for the values between the time intervals (2 weeks) ($p<0.01$) for salivary MDA in children with Down syndrome with dental caries (Table 1). This can be attributed to the fact that dental caries is a multifactorial inflammatory disease. The inflammatory response in dentin due to caries is mainly due to oxidative stress leading to the destruction of dental hard tissues which initiates caries process (Subramanyam D *et al.* 2018). Also, it causes alterations in immune responses of saliva leading to impaired salivary peroxidase antioxidant systems (da Silva PV *et al*. 2016). These results are similar to the findings of studies conducted by Tulunoglu et al., 2006; Hegde et al., 2009; Preethi et al., 2010; Dodwad et al., 2011; Kumar et al., 2011; Ahmadi-Motamayel et al., 2013; Mahjoub et al., 2014 which showed significantly higher levels of Total Antioxidant Capacity in dental caries.

However, the findings of our study are at variance from results obtained by Rai et al. who studied the relation between lipid peroxidation and dental caries and found out that there was no difference in salivary MDA levels in normal children with or without Early Childhood Caries. Also, Oztürk et al. compared the association between DMFT and salivary MDA levels in the dental caries, and they did not find any significant difference in salivary MDA levels among the groups studied.

In the present study, the levels of oxidative stress decreased significantly in patients

following rehabilitation. It may be due to increased production of salivary antioxidant enzymes post interventions. Salivary peroxidase catalyses the peroxidation of the thiocyanante ion to generate oxidation products that inhibit the growth and metabolism of many microorganisms thus, slowing down the caries progression and inflammation post treatment (Öztürk LK et al. 2008). This is in concordance with the study done by Menon et al. on inflammatory biomarkers in which they have showed the elevated levels of Interleukin-6 in early childhood caries got reduced after treatment.

Also, Varma et al assessed the levels of Interleukin-1β in children with Down syndrome after full mouth rehabilitation and found that the levels of Interleukin-1β significantly decreased after treatment. From the above-mentioned studies, it is clear that there is a strong correlation between levels of these inflammatory mediators and the presence of dental caries in special children and their levels decreased after rehabilitation.

However, in children with Down syndrome with dental caries no statistically significant difference was seen for salivary pH. This was similar to the findings obtained by Bassoukou et al, and Rai et al who did not find any statistically significant differences in salivary pH. It was mainly due to the reason that saliva buffering capacity works by counteracting the decrease in pH and is an important protective factor against caries.

In the present study, statistically highly significant difference was seen in children with Down syndrome with dental caries for the values ($p<0.01$) of salivary viscosity, with higher values seen at pre than post treatment of carious lesions (Table 3). This is in concordance with studies conducted by Kaur et al and Animireddy et al where relative viscosity of saliva in children belonging to the caries-active group was significantly ($P < 0.0001$) greater than relative viscosity of saliva in children belonging to the caries-free group. Greater the viscosity of saliva, lesser will be its cleansing action leading to a higher caries

rate.

Buffering capacity of Down syndrome children have been reported to be higher, similar to the findings of our study where intergroup group comparisons showed higher values in Down syndrome children, however it was statistically non-significant (Table 4). This was mainly attributed to higher phosphorus levels in their saliva. This is similar to the findings of studies conducted by Siqueira et al., where buffering capacity was found to be high in Down's syndrome children compared to control group. On the contrary, Yarat et al. worked with individuals aged 7–22 years, found no difference in the buffer capacity comparing the Down syndrome and control. They suggested a relationship between the low dental caries prevalence and the high concentration of bicarbonate which have been found in children with Down syndrome.

In our study, there was a statistically highly significant difference seen for the values ($p<0.01$) of buffering capacity in children with Down syndrome with dental caries where higher values were seen at Post as compared to Pre intervention group (Table 4). This may be due to the reason that the buffering capacity of both stimulated and unstimulated saliva acts by involving three major buffering systems, namely the bicarbonates, phosphates and proteins, therefore it facilitates neutralization of acids produced by bacteria in the oral cavity, thereby slowing down the progression of caries post treatment (Sruthi K S et al.2019).

This is in accordance to the results obtained by Prabhakar et al. in 2009 and Preethi et al. in 2010. However, the results obtained in their studies were not statistically significant. The salivary buffering capacity was only slightly reduced in caries-active children compared with caries-free children. Another study done by Zhou et al. in 2007 showed that the buffering capacity of saliva from early childhood caries children was statistically higher than that in caries-free children. A study performed by Malekipour et al. in 2008 showed similar

results, although the difference was not statistically significant.[30]

Hence, the levels of MDA, salivary pH, viscosity and buffer capacity significantly decreased post treatment of carious lesions in children with Down syndrome. Thus, indicating that undergoing caries control treatment helps to reduce their oxidative stress. With various rehabilitative interventions, it helped in minimizing the levels of oxidative stress among these children, thus bringing down the severity of their disease.

Therefore, evaluation of these biomarkers in children with Down syndrome helps in accurate caries risk evaluation and improved treatment efficacy, which will improve overall oral health status of these children.

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