Biomedical & Pharmacology Journal, September 2018. Vol. **11**(3), p. 1565-1571

Evaluation of Reactive Oxygen Metabolites in Down Syndrome Persons With Periodontitis— A Comparative Study

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http://dx.doi.org/10.13005/bpj/1523

(Received: 31 May 2018; accepted: 13 September 2018)

Down syndrome (DS) is one of the most common chromosomal disorders, occurring in one out of 700-1000 live births, and the most common cause of mental retardation. Increasing evidence has shown that DS individuals are under unusual increased oxidative stress, which may be involved in the higher prevalence and severity of a number of pathologies associated with the syndrome, as well as the accelerated ageing observed in these individuals. ROS leads to oxidative damage of DNA, proteins and lipids; therefore, oxidative stress may play an important role in the pathogenesis of DS. Periodontal disease is a common problem among DS individuals. The disease starts early in life and progresses with age eventually leading to tooth loss. This study was undertaken to evaluate and compare the levels of ROM in DS subjects with periodontitis and systemically healthy subjects with chronic periodontitis. There is significant increase in levels of ROM and shows statistically significant value (<0.001) in DS with Chronic periodontitis patients.

Keywords: DS- Down Syndrome, ROM- Reactive Oxygen Metabolites, ROS- Reactive Oxygen species, SOD- superoxide dismutase.

The balance between the oxidation and anti oxidation is essential for the system homeostasis. Reactive oxygen species (ROS) are formed under physiologic state in which imbalance in ROS concentration which causes series of radical reaction which further leads to degradation of macronutrients such as proteins lipids and sugar. When imbalance occurs in reactive oxygen species it leads to oxidative stress, invariably causing cell damage and cell death [1].

Within the gingival sulcus or the periodontal pocket, the bacteria are not intracellular pathogens (unlike viruses) and therefore maintaining a low redox state within a cell may not have relevance in comparison to a high redox state.

Reactive oxygen metabolites and free radicals form in various sources which includes endogenous as well as exogenous sources. Host defense cells and connective tissue cells are the endogenous sources wheras trauma, infections, smoking, therapeutic drugs, heat are the forms of exogenous source.

Damage to the extracellular tissue and cellular tissue caused by ROS which further leads to damage to proteins and DNA and lipid peroxidation.

Down syndrome (DS) is a common chromosomal abnormality which in turns leads

to third copy chromosome 21. The Gene dosage effect (variations in molecular bases) which leads to imbalance on gene expression, though molecular mechanisms by which such gene dosage imbalance is still unclear.[2]

Extra copy of chromosome 21 which occurs in one in a 1000 births also increases in occurrence with age of the mother. 80% of the children with DS are born to women above 35years of age.[3] John Langdon Down in 1866 who so called as father Down syndrome who first reported the clinical symptoms but extra copy of Chromosome 21 was first described by Lejeune. [5] Only 5- 10% DS are caused by the other genetic abnormalities (chromosomal translocations and mosaicism) and about 90-95 % is due to Trisomy 21.[6,7]

"Gene dosage effect" which is due to the imbalance on gene expressions and this is caused by the third copy of chromosome 21 but molecular mechanism behind this is still unclear. [2]

Down syndrome persons are more susceptible to infections and other systemic diseases like leukemia , endocrine disorders, nutritional disturban ces etc. Literatures has been suggested that ROM is caused by the mutation in gene encoding which excessively increase SOD levels which is responsible for initiation of pathogenesis in DS.[8]

Trisomy 21 had high SOD1 expression and activity which led to an interenzymatic imbalance in the antioxidant defense system. [9]

With the known fact the role oxidative stress has a vital role in initiating the pathogenesis in Ds subjects. The aim of the present study to estimate the levels of ROM in Down's syndrome persons with chronic periodontitis and compared with healthy person with chronic periodontitis and without chronic periodontitis.

Inclusion criteria

1) Systemically healthy subjects with healthy periodontium with probing pocket depth < 3mm
2) Systemically healthy subjects with Chronic periodontitis with probing pocket depth > 5mm in more than 30% of sites
3) Down's syndrome subjects with Chronic periodontitis with probing pocket depths measuring >5mm in more than 30% of sites

Exclusion criteria

1) Patients having less than 14 teeth
2) Patient with other systemic diseases
3) Patients with co-existing oral infections
4) Smokers
5) Patients on antibiotic therapy six months prior to study
6) Patients who had received periodontal treatment six months prior to the study

MATERIAL AND METHODS

Total 60 subjects with same age group were categorized into three groups;

Group I - Systemically healthy subjects without periodontitis

Group II- Systemically healthy subjects with periodontitis with probing pocket depth > 5mm in more than 30% of sites

Group III- comprising of subjects with Down's syndrome (ds) with periodontitis with probing pocket depth > 5mm in more than 30% of sites

Clinical examination

Age, Sex, habit, past medical history, past dental history and following clinical parameters were taken.

Clinical Parameters (by green and vermillion)

1. Plaque Index
2. OHI-S (oral hygiene index simplified
3. CPITN (Community periodontal index for treatment need by ainamo)

Samples collection

Ethical committee clearance obtained from Dr. MGR Educational and Research Institute. Systemically healthy subjects with and without periodontitis samples were taken from out patients attending department of Periodontics in Thai Moogambigai Dental College and Hospital and Down's syndrome subjects with periodontitis were taken from Various institutes for down's syndrome in Chennai, consent form dually signed by parents and care takers of the home. CPITN Probe (hufirdy) was used measure the periodontal Index 5ml un stimulated pooled saliva collected from the patients and samples are processed immediately using to detect the ROM levels

Draining / Spitting method

Patient has to collect **u**nstipulated saliva in the floor of mouth and then spit in to sterile container.

Laboratory method for detection of ROM

The d- ROM test, developed by world renowned Italian biochemist Mauro Carratelli, is a photometric test for measurement of the concentration of hydroperoxides (ROOH) in biological samples. The presence of ROOH in cells indicates oxidative attack of ROS on various substrates such as carbohydrates, lipids, amino acids, proteins, or nucleotides.

The measurement of salivary ROM level was performed using a UV-visible spectrophotometer. Test evaluates its ability of transition metals to catalyze, in the presence of peroxidase the free radicals which are trapped by alkalamine. 20µl of saliva and 1 ml of acetate buffer with that 20 ul chromogenic substrate was added. After mixing, the cuvette was immediately incubated in the thermostatic block of the analyzer for 5 minutes at 37°c. The measurement unit was expressed as carratelli unit (CARRU). It has been established that (CARRU) corresponds to 0.08 mg/dl hydrogen peroxide.

RESULTS

All 60 Patients attended there was significant difference in ROM. Subjects included in our study were in the age group of 12 to 30;

Table 1a. Comparison of mean Age between the three groups using oneway ANOVA

Groups	N	Mean	Std. Deviation	Minimum	Maximum	P value
DS+CP	20	18.65	6.99	12.00	35.00	
CP	20	25.95	2.52	22.00	30.00	<0.001*
HEALTHY	20	24.15	2.36	20.00	29.00	
Total	60	22.91	5.42	12.00	35.00	

*P<0.05 is considered statistically significant

Table 1b. Post hoc multiple comparisons using Tukey HSD test

(I) Group	(J) Group	Mean Difference (I-J)	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
DS+CP	CP	-7.30*	0.000	-10.7271	-3.8729
	HEALTHY	-5.50*	0.001	-8.9271	-2.0729
CP	DS+CP	7.30*	0.000	3.8729	10.7271
	HEALTHY	1.80	0.421	-1.6271	5.2271
HEALTHY	DS+CP	5.50*	0.001	2.0729	8.9271
	CP	-1.80	0.421	-5.2271	1.6271

To Assess with Plaque index score DS with chronic periodontitis subjects have more significant p value <0.001

Table 2a. Oneway ANOVA for Plaque index

Groups	N	Mean	Std. Deviation	95% Confidence Interval for Mean		Minimum Bound	Maximum	F	P value
				Lower	Upper Bound				
DS+CP	20	2.2520	0.65043	1.9476	2.5564	1.00	3.00		
CP	20	2.2000	0.61559	1.9119	2.4881	1.00	3.00	42.8	<0.001
Healthy	20	0.6875	0.55200	0.4292	0.9458	.00	2.00		
Total	60	1.7132	0.94433	1.4692	1.9571	.00	3.00		

however in the Down syndrome groups the patients, the patients were younger, with a mean age of 18.65. This in when compared with the other groups was statistically significant $p < 0.001$, when utilizing the One Way ANOVA indicating that the Down Syndrome group developed periodontal breakdown, leading to chronic periodontitis at an earlier age. The difference in age when compared utilizing the Tukey HSD Test, to compare individual groups to the Down syndrome group revealed a highly significant difference in the incidence of chronic periodontitis in this group

To further assess the presence of local factors that contribute to periodontal destruction

Table 2b. Inter group comparisons Post HOC test for Plaque index

(I) grp	(J) grp	Mean Difference (I-J)	Sig.	95% Confidence Interval Lower Bound	Upper Bound
DS+CP	CP	.05200	0.960	-.4102	.5142
	Healthy	1.56450*	<0.001	1.1023	2.0267
CP	DS+CP	-.05200	0.960	-.5142	.4102
	Healthy	1.51250*	<0.001	1.0503	1.9747
Healthy	DS+CP	-1.56450*	<0.001	-2.0267	-1.1023
	CP	-1.51250*	<0.001	-1.9747	-1.0503

Table 3. OHI-S among all three groups compared using Chisquare test

			Group DS+CP	CP	Healthy	P value
OHI_S	GOOD	Count	5	0	0	
		% within OHI_S	100.0%	.0%	.0%	<0.001*
	FAIR	Count	3	13	18	
		% within OHI_S	8.8%	38.2%	52.9%	
	POOR	Count	12	7	2	
		% within OHI_S	57.1%	33.3%	9.5%	
Total	Count		20	20	20	
	% within OHI_S		33.3%	33.3%	33.3%	

*P<0.05 is considered statistically significant

Table 4. CPITN in all three groups compared using Chisquare test

		Group DS+CP	CP	Healthy	P value
CPITN	No Rx	0	0	8	
		.0%	.0%	100.0%	<0.001*
	TN 1	3	5	9	
		17.6%	29.4%	52.9%	
	TN 2A/B	8	10	3	
		38.1%	47.6%	14.3%	
	TN 3	9	5	0	
		64.3%	35.7%	.0%	
Total	20	20	20		
	33.3%	33.3%	33.3%		

*P<0.05 is considered statistically significant

the Oral Hygiene Index was performed on the examined patients. The Down's syndrome patients showed a statistically significant increase in the OHI score which was statistically significant, when assessed using the Chi Square Test.

To understand the periodontal health of the patients, the CPITN was utilized. The Down's syndrome patients had a statistically significant increase in the probing sulcus depth

The level of Reactive Oxygen metabolites in the groups was assessed with the CARRATELLI Units (CAARU levels). The Down syndrome patients revealed a mean CAARU score of 753.2; whereas the chronic periodontitis among systemically healthy patients had a mean score of 482.15. The periodontally healthy patients, had a mean CAARU score of 334.35. This difference in the levels of Reactive Oxygen Metabolites was statistically significant, was statistically significant ($p< 0.001$).

When the individual groups were compared using the Post Hoc Tukey Test, the levels of Reactive Oxygen metabolites, revealed a highly significant Difference in the Down Syndrome group when comparing individually with the Chronic Periodontitis in systemically healthy patient group and when compared with the Healthy Periodontium group. The level of significance in these inter-group comparisons were all statistically highly significant ($p<0.000$)

Statistical evaluation

The statistical software SPSS version 17.0.0 (SPSS manufacturer IBM Corporation, 1 New Orchard Road, Armonk, New York 10504-1722, United States) was used for the analysis of the data. $P < 0.05$ is considered level of significance

Each study group was estimated the mean and standard deviation and their mean values were evaluated with Anova and post hoc test.

DISCUSSION

This was the first epidemiologic study assess salivary ROM levels in Down's syndrome patients and to show that salivary oxidative status was positively associated with periodontal conditions increase in the OHI, CPITN score compared with systemically healthy and We have compared with the multiple groups was statistically significant $p < 0.001$,when utilizing the One Way ANOVA indicating that the Down's Syndrome group developed periodontal breakdown, leading to chronic periodontitis at an earlier age. In our study Plaque index was statistically significant

Table 5a. Comparison of mean CAARU among the three groups using oneway ANOVA

Groups	N	Mean	Std. Dev	Min	Max	P value
DS+CP	20	753.20	265.03	269.00	1181.00	
CP	20	482.15	190.99	273.00	895.00	
HEALTHY	20	334.35	107.50	217.00	656.00	
Total	60	523.23	262.07	217.00	1181.00	<0.001*

*P<0.05 is considered statistically significant

Table 5b. Post Hoc multiple comparisons using Tukey HSD test

(I) Group	(J) Group	Mean Difference (I-J)	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
DS+CP	CP	271.05*	0.000	119.9501	422.1499
	HEALTHY	418.85*	0.000	267.7501	569.9499
CP	DS+CP	-271.05*	0.000	-422.1499	-119.9501
	HEALTHY	147.80	0.056	-3.2999	298.8999
HEALTHY	DS+CP	-418.85*	0.000	-569.9499	-267.7501
	CP	-147.80	0.056	-298.8999	3.2999

(<0.001) in systemically healthy subjects with Chronic periodontitis group and DS subjects with chronic periodontitis group, when assessed using the Chi Square Test. Further clinical parameters was evaluated in which Down's syndrome with Chronic periodontitis patients have significant systemically healthy with chronic periodontitis group. The results of our present study which compared with *Ana Cristina Amaral Loureiro in 2007*.[10] who also compared the clinical parameters which shows the similar results that Down's syndrome subjects definitely have more negative impact on periodontium when compared with systemically healthy patients this may be because of Patients with DS they have poor IQ levels and often institutionalized they are unable to maintain their oral hygiene measures on their own.

ROM is considered to be a reliable indicator for oxidative status, Logistic regression analysis also showed that the subjects with ROM >400 CARRU had significantly higher, In these results suggested that the higher oxidative status of saliva could have affected the rate of progression of periodontal disease and it is also have a effect on periodontium.[11]

The ROM was assessed with the CARRATELLI Units (CAARU levels). The Down syndrome patients revealed a mean CAARU score of 753.2; whereas the chronic periodontitis among systemically healthy patients had a mean score of 482.15. The periodontally healthy patients had a mean CAARU score of 334.35. The present study results indicates the difference in the levels of Reactive Oxygen Metabolites and values are statistically significant ($p< 0.001$) the results of our study correlated with the study done by *Kedziora J* in 1988.[12] He states that initiation of pathogenesis in down syndrome may be because of imbalance in ROM levels.

Unusual oxidative stress happened in Down syndrome a subject which is due to excess of Cu/Zn superoxide dismutase (SOD1), an enzyme coded on HSA2. [13,14] Hydrogen peroxides (H2O2) is an important originators of hydroxyl radical which has been enhanced by SOD1, 16 genes on HSA21 which has a role in mitochondrial energy generation and ROS metabolites.[15]H2O2 is then neutralized to water and oxygen through the actions of either glutathione peroxidase (GPx) and/or catalase (CAT). Hence, the increased ratio of SOD1 to catalase plus glutathione peroxidase can lead to increased oxidative stress in DS. [16]

However, in our study, the periodontal condition correlated with salivary oxidative stress, since the presence of other local factors for progression of periodontal disease was present and oxidative stress may be involved in the initiation of pathogenesis of periodontal diseases. [17, 18]

The association between our results and the groups were categorize by the diagnosis of periodontal disease with significant increase in ROM levels in Down's syndrome with chronic periodontitis compared with systemically healthy chronic periodontitis and systemically healthy controls this has to be taken in to consideration that increase in ROM levels which not only give negative impact on periodontium also to in general health of the subjects

CONCLUSION

Our present study results reveals the significant increase in reactive oxygen metabolites which has negative impact on periodontium on Down syndrome children, Further studies needed to evaluate post treatment ROM levels in Down's syndrome children

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