Correlation Between Flow Rate, Viscosity, Buffering Capacity, pH and Carries in Full and Mozaic Down Syndrome Children: A Study in Trisomy and Mozaic Type Down Syndrome

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Abstract

Down Syndrome (DS) is a genetical abnormality caused by the abnormal amount or structural chromosome 21. Children with DS are in/have a higher risk of dental caries that related to salivary composition. The aim of the study was to analyze the difference of flow rate viscosity, buffer capacity, salivary pH with caries in children with full trisomy and mosaic type DS and its correlation with caries.

This was an analytic correlational design study. The subject was 15 DS children aged 5-18 years, consisted of 3 mosaic type and 12 full/ complete trisomy type. The research used nonstimulation of the saliva. The salivary flow rate was measured by the amount of collected saliva during 5 minutes, divided by the time to collect the saliva. Salivary viscosity was visually measured based on the amount of the bubbles and the flow ability when the glass was moved to leaning position, Buffering capacity was recorded through the scores of color changes on the test pad. Salivary pH was scored based on color changes of the strip test. Caries was analyzed using DMFT index and deft. Statistical differentiation test using U Mann-Whitney and Kendal Rank Correlation test to find out the correlation of salivary function and the occurrence of caries.

The results showed there were significant differences of flow rate, viscosity, buffering capacity, salivary pH and the occurrence of caries between children with full trisomy and mosaic type DS, besides significant correlation between flow rate, viscosity, buffering capacity, salivary pH and dental caries in children with full type DS and slight correlation in mosaic type.

It was concluded that there were significant differences of flow rate, viscosity, buffering capacity, salivary pH and carries between children with full trisomy and mosaic type DS.

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Introduction

Down Syndrome is a kind of genetical abnormality the caused by abnormal amount or structure of chromosome 21. This abnormality occurs when a pair (a set) of chromosome 21 fail to set apart during division. The change in the amount or structure of chromosome 21 in the DS might affect the genetic balance of the body and results as special characteristic physical, intellectual ability and physiology physical abnormality of function[1-3].

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DS that is caused by an abnormality of the chromosome amount consists of full trisomy and mosaic type. In full trisomy type, all of the cells in the body have extra chromosome 21, while in mosaic type, there is a combination of normal cells and extra chromosome 21[3,5]. The prevalence of DS is different in every country, even in every area within a country. According to WHO, the incidence of DS is globally about 1-10 in 1000 live births[6]. In Indonesia, according to Riskesdas (2013), the incidence was 1.3 in 1000 live births[7].

DS children are those with special needs and are at risk to have dental caries that caused by difficulties in oral hygiene, muscle weakness, and inability to move the muscle that affects the routine procedure in cleansing the teeth[8]. Several studies revealed that DS children had lower caries index compared with non-DS children, in

spite several studies found out no differences while other studies revealed that DS children/individuals had higher caries index[9-12].

Saliva is an exocrine liquid that is existed into the mouth cavity by salivary gland[13]. The secretion of saliva is managed is controlled by the neurological system, the sympathetic as well as parasympatic[14]. Genetical abnormality in the DS patient might cause abnormality of the neurological system that results from an abnormality of the sympathetic and parasympathetic nerves and cause changes in salivary secretion. Neurological abnormality in the DS as a combination of abnormal development and functional changes the caused neurological abnormality that accompanies DS as the effect of gene expression (exaggerated) in the trisomy cellsRCAN1 gene locus on 21q22.12, DYIRKIA on 22q22.12 locus, and DSCAM on locus 21q22.2 that genes are located on DSCR (Down Syndrome Critical Region) chromosome 21 that Significantly correlated with characteristic abnormality of the neurological system in DS[15].

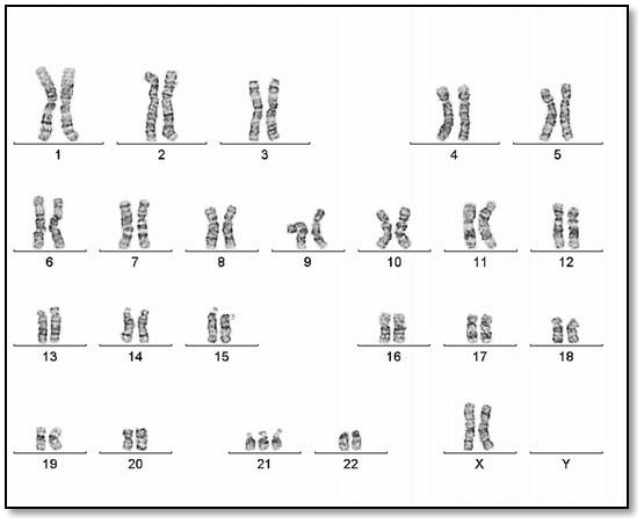

Figure 1. Genetic Analysis For Trisomy 21 Diagnosis[4].

The Composition of the saliva that MIGHT have related to the occurrence of caries is Among others flow rate, viscosity, buffering capacity and salivary pH[13,16,18]. High viscosity will Decrease the salivary flow rate and self-cleaning ability of the saliva, and accumulate in the mouth cavity that Becomes the main cause of caries[19]. The salivary flow rate and buffering capacity support each other in Preventing and turn back the demineralization process in the beginning of caries lesion. The high flow rate of the saliva makes the cleansing effect faster and increases the buffering capacity of the saliva[16,20,21].

Buffering capacity of the saliva has a role in the salivary pH acidity when acidic conditions occur.The Increase of the salivary pH will Facilitate the remineralization process on the email and dentin surface, and Also Prevent acid formation by microorganisms[21-23].

A study by Castillo and Areias revealed that the Decrease of flow rate and buffering capacity of the saliva accompany (accompanying) the high index of caries in DS children[10,12,24,25]. A study by Raurale and Yazeed Reported that the low index of caries in DS children was accompanied by the increase of odd buffering capacity, salivary pH, and Decrease of salivary viscosity[9,24]. It was Also Reported that a salivary gland in DS children had a change on ducts and acinar cells[26-28].

The aim of this study was to analyze the difference of flow rate, viscosity, buffering capacity, salivary pH with the occurrence of caries in children with full trisomy and mosaic type DS and its correlation with the occurrence of caries.

Materials and methods

A sample of the study.

The subject of this study were DS children whose chromosome were examined with full and mosaic type trisomy. Sample collection by purposing sampling.

Inclusion Criteria

Down Syndrome children aged 5-18 years.

Exclusion criteria

1. DS children who were not cooperative.
2. DS children with agenesis dental

Materials

In this study, data collecting were using

1. Saliva-Check Buffer kit – CG
2. Diagnostic tools, mirror, sonde, tweezers.
3. Stopwatch

Figure 2. Research Tools.

Methods

This was a correlational analytic designed study.

Operational Definition

1. DS child is a patient diagnosed with Down Syndrome, full or mosaic type trisomy.
2. The flow rate is the amount of not-stimulated saliva in the mouth cavity that is spat out into a measuring glass every minute for five minutes.
3. Salivary viscosity means the consistent condition of non-stimulated saliva, visually measured by moving the filled measuring glass, then the flow of the saliva is measured and count the visible bubbles.
4. Buffering capacity of the saliva means the ability of saliva in neutralizing the acid, measured by Saliva-Check-Buffer kit GC. The saliva was taken from the measuring glass using a pipette, then one by one drop was placed on each test pad of buffer test strip. The score of buffering capacity was counted by adding the score of the color change on each test pad two minutes later with pointed unit 0-12 (Figure 3., Table 3 and Table 4). Data collected were on an ordinal scale.
5. Salivary pH means a grade of acidity measured using pH paper mark CG, pH paper strip is dyed for 10 seconds into measuring glass filled with saliva. The score of salivary pH is counted by adjusting the color on the pH paper strip when the paper strip is still wet and the indicator of salivary pH as Shon in Figure 4 and Table 5. The data were on an ordinal scale.
6. Dental caries is the cavity on the dental surface based on sonde plugs. Dental caries is measured (counted) using DMF-T index for permanent teeth and def-4 for deciduous teeth.

1) DMF-T for permanent teeth:
   1. D (decay): teeth with caries
   2. M (missing): indicated by caries
   3. F (filing): number of teeth filled patched
   4. Index DMF-T = $\frac{\text{DMF teeth}}{\text{Number of patient examined}}$

2) def-t for deciduous teeth
   1. d (decay): number of carriers filled
   2. e (extraction) number of caries teeth indicated to be extracted
   3. f (filing): number of teeth filled and in good condition.
   4. def index: def-t index: $\frac{\text{def teeth}}{\text{Number of patient examined}}$

3) Category DMF-T and def-t according to World Health Organization.
   1. 0.0-1.1 = Very low
   2. 1.2-2.6 = Low
   3. 2.7-4.4 = Moderate
   4. 4.5-6.5 = High
   5. >6.6 = Very high

Score	Category	Expl.
1	Slow	<0.7 mL/minute
2	Normal	0.7-1 mL/minute
3	Fast	> 1 mL/minute

Table 1. The Criteria of Flow Rate[29].

Score	Category	Expl.
1	Low	The saliva was looked liquid like, stagnate, no bubble
2	Medium	The saliva was looked white, bubbled, does not stagnate, frosty bubbly
3	High	The saliva was sticky, white colored, Bubbly, frothy.

Table 2. Criteria Of Salivary Viscosity[29]

Figure 3. Buffering capacity test pad of the saliva Quoted from GC Corporation[29]

Score	Color
4	**Green**
3	**Partial blue/green**
2	Blue
1	Partial blue/red
0	Red

Table 3. Test Pad Score Of Color Changes After 2 Minutes[29].

Total point	Buffering capacity of saliva
0-5	Very low
6-9	Low
10-12	Normal

Table 4. Interpretation Of The Last Buffering Capacity Of The Saliva[29]

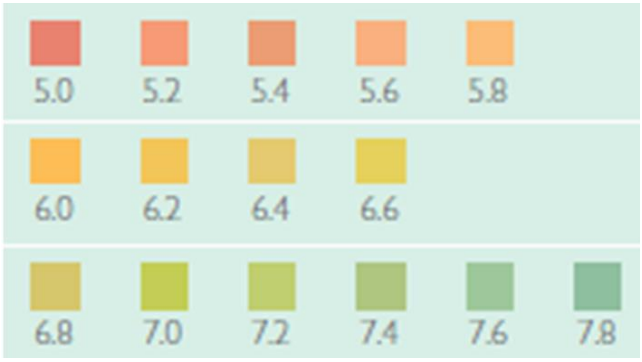

Figure 4. An indicator of salivary pH Quoted from GC Corporation.[29]

Color	pH	Expl.
Red	5,0-5,8	Low
Yellow	6,0-6,6	Medium
Green	6,8-7,8	High

Table 5. Interpretation Of Salivary pH[29].

Research/Study Procedure

The research/study procedure included:

1. Ethical clearance
2. Collecting subject according to the inclusion criteria.
3. Filling informed consent.
4. Examination of salivary flow rate:
   1) The subject did not take/eat any food or drink for about one hour before salivary taking.
   2) The subject was asked to sit comfortably, then gurgle using aqua dest.
   3) Subject was asked to lower the head and did/made as minimal movements such as speaking
   4) Spit out the saliva in the mouth cavity into the measuring glass every minute for five minutes
5. Salivary viscosity examination:
   1) Put the filled measuring glass in lean position.
   2) Visually examine the salivary flow to find out the flowability and number/amount of bubbles.
6. Examination of buffering capacity.
   1) Take out the buffer set strip from the aluminum package and put it down on a tissue paper.
   2) Take a little amount of saliva using saliva dispensing pipette
   3) Pour down a drop of saliva on each pad of buffer test strip.
   4) Learn the buffer test strip at 90* position to make the rest of saliva flow to the tissue.
   5) Check the color change on each pad of the buffer test strip after 2 minutes.
7. Measuring salivary pH.
   1) Collect the saliva in saliva dispensing cups
   2) Drown (dye) the pH test strips for 10 seconds into the saliva dispensing cup.
   3) Compare the colors on each pH test strip with the color on the table to consider salivary pH score when the pH test strips are still wet.
8. Examination of dental caries.

The Intraoral examination was done on each quadrant using mouth mirror and sonde, then count the broken teeth, missing, or indicated resources to be extracted, and filled teeth.

Results

Examination of the flow rate, viscosity, buffering capacity, salivary pH, and occurrence of dental caries was done on 15 DS children aged 5-18 years and the results are shown in tables 6, 7, 8, 9, 10, and 11.

Sample	Flow Rate:	
	Full trisomy	Mozaic
1	Slow	Slow
2	Slow	Slow
3	Slow	Normal
4	Slow	
5	Slow	
6	Slow	
7	Slow	
8	Slow	
9	Slow	
10	Slow	
11	Slow	
12	Slow	

Table 6. Results Of Salivary Flow Rate Examination On DS Children With Full Trisomy And Mosaic Type. P<,05.

Sample	Viscosity	
	Full Trisomy	Mozaic
1	Moderate	Moderate
2	High	Low
3	Moderate	Low
4	High	
5	Moderate	
6	Moderate	
7	High	
8	High	
9	High	
10	Moderate	
11	High	
12	High	

Table 7. Results Of Salivary Viscosity Counting On DS Children With Full Trisomy And Mosaic Type. P<,05.

Sample	Buffering Capacity	
	Full trisomy	Mozaic
1	Very low	Normal
2	Very low	Normal
3	Low	Low
4	Low	
5	Low	
6	Very low	
7	Very low	
8	Very low	
9	Very low	
10	Low	
11	Low	
12	Low	

Table 8. Results Of Buffering Capacity Measuring In DS Children With Full Trisomy And Mosaic Type. P<,05.

Sample	pH	
	Full trisomy	Mozaic
1	Moderate	High
2	Low	High
3	Low	Moderate
4	Low	
5	Moderate	
6	Low	
7	Low	
8	Low	
9	Low	
10	Moderate	
11	Moderate	
12	Moderate	

Table 9. Results Of Salivary Ph Measuring On DS Children With Full Trisomy And Mosaic Type. P<,05.

Sample	DMF amount	
	Full trisomy	Mozaic
1	4	2
2	5	2
3	2	6
4	4	
5	3	
6	4	
7	4	
8	5	
9	8	
10	3	
11	9	
12	7	

Table 10. Results Of DMF Measuring On DS Children With Full Trisomy And Mosaic Type. P<,05.

Sample	def amount	
	Full trisomy	Mozaic
1	8	3
2	15	2
3	13	0
4	9	
5	8	
6	10	
7	8	
8	10	
9	0	
10	0	
11	0	
12	2	

Table 11. Results Of Def-Counting In DS Children With Full Trisomy And Mosaic Type. P<,05.

Discussion

The study subject was 15 children, consisted of 12 full trisomy and 3 mosaic types. Based on the chromosomal abnormalities, 80% were full trisomy and 20% mosaic types. Cytogenetic examination on DS patients showed that full trisomy type is most often has a chromosomal abnormality (93-96%), translocation type (2-5%), and mosaic type were about 2-3% of global DS cases[2]. The prevalence of the mosaic type in this study was higher compared with the results of other studies and was in accordance with an epidemiologic study on DS patients in India by Chandra et al[30]. Mozaic type DS with more than 40% extra chromosome cells was diagnosed as full trisomy, and those of less than 10% were sometimes not diagnosed. Therefore, the reported frequency of mosaic type on DS cases was lower than the real frequency[31].

Nonstimulated saliva has an important role in the health of oral cavity, the saliva is a strong protection against dental caries[16]. Salivary flow rate in the majority of the samples was 93.37% of the slow category and the rest 6.6%were in the normal category. This result was in accordance with a study by Castilho et al., Raurale et al., Davidovich et al., Asokan et al., and Franco et al., which stated that salivary flow rate in those (patients) with DS decreased[12,24,25,32-34].

Siqueira et al. revealed /found out that the decrease in salivary flow rate in DS children was caused by the changes of metabolites of ducts and acinar cell of salivary glands, while Areias et al stated that it was caused by functional changes in salivary gland secretion as results of developmental abnormalities of the salivary gland[26,28,34]. The decrease in salivary flow rate is caused by more expression of RCAN, gen on the locus 21q22.12, DYRKIA on locus 21q22.13, and DSCAM on locus 21q22.2 which are genes that were located on Down Syndrome Critical Region (DSCR) of chromosome 21. GensRCANI, DYRKIA, and DSCAM are strongly related with characteristic neurological system disorder in Down Syndrome. Secretion of salivary gland affects the composition of saliva that might result/cause changes in the composition of the saliva [13,16,18].

The high salivary viscosity may cause retention of food on the teeth surface that may

increase the risk of dental caries. The results showed that salivary flow rate was slow. This was in accordance with the high salivary viscosity (46.7%).

The result of buffering capacity of the saliva showed that most of the subjects were in low category (46.7%). Buffering capacity of the saliva is likely affected by bicarbonate ion that is resulted from cell metabolisms. The concentration of bicarbonate ion will increase the salivary flow rate also increases. The low salivary flow rate is in accordance with low buffering capacity.

Salivary pH in DS children in this study was mostly low (46.7%) and there was a significant difference between full trisomy and mosaic type. This was in accordance with the study by Normastura et al, that found the salivary pH were acid[35].

The full trisomy group had a high category of DMFT index and very high def-t category, while. the mosaic group had lower (moderate) DMFT index and low index deft category. Dental caries is a multifactorial disease that is affected (influenced) not only by dietary food but Also the host factor[17].

Analysis of the saliva including flow rate, viscosity, buffering capacity, pH, showed a significant difference between full trisomy and mosaic group ($p<0.05$). The results were in accordance with the study by Winer and Feller on the salivary composition of full trisomy DSS, translocation, and mosaic, that mosaic type DS had a higher flow rate, pH, bicarbonate, calcium, sodium, and chloride[36]. The result of the different test on the caries occurrence between full trisomy and mosaic DS children showed significant differences. On mosaic type DS there were extra chromosome 21 on less than 40% body cells. Body cells with lower extra chromosome 21 might result in/might cause the mosaic type DS had physically or mentally better phenotype, compared with full trisimy[4]. The development process of caries is affected by the dental structure. Email hypoplasia is an anomaly of the dental structure as a manifestation of oral DS and increases the susceptibility towards carries. The lower percentage of cells with extra chromosome 21 in mosaic-type results in a better dental structure that is stronger against caries.

The result of correlated analysis between flow rate, viscosity, buffering capacity and salivary pH with the occurrence of caries on trisomy group revealed a significant correlation, while in the mosaic group the correlation was insignificant.

Conclusions

There was a significant difference of flow rate, viscosity, buffering capacity, salivary pH and carries between DS children with full trisomy and mosaic type. There was a stronger correlation of flow rate, viscosity, buffering capacity and salivary pH with caries occurrence between DS children with full trisomy compared with mosaic type.

Declaration of Interest

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