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Six Minutes Wonder: The effect of 6-minute-walk test on salivary parameters in children

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ABSTRACT:The blood-line of the oral cavity with its numerous components protects and maintains health and optimal functioning of the oral hard and soft tissues. The current study was undertaken to assess the effect of 6-minute-walk-test (6MWT) on salivary pH, flow rate and amylase in children. Children between 5 and 11 years of ageattending the out-patient department with no prior dental treatment were randomly selected and the 6MWT was conducted according to guidelines given by the American Thoracic Society (ATS). Unstimulated salivary samples were collected and then the children were asked to undertake the 6MWT following which a second sample of unstimulated saliva was collected. Both the pre-test and post-test samples were assayed for pH, flow rate and amylase. Statistical analysis was done using paired t-test to compare the pre- and post- test values and evaluate any changes in the three salivary parameters. pH showed an alteration towards alkalinity from a mean of 6.97±0.15 to a mean of 7.24±0.16. Salivary flow rate enhanced from a mean of 0.28±0.05ml/min to a mean of 0.41±0.11 ml/min. Amylase levels significantly improved from a mean of 32.89±17.31 mU/min to a mean of 62.43±21.48 mU/min.The results of the current study show that 6MWT has a positive shift in salivary defence dynamics among children signifying the importance of sub-maximal physical activity in maintaining good oral health.

KEYWORDS:6-minute-walk-test, Salivary amylase, Salivary pH, Salivary flow rate, Physical activity in children.

I. INTRODUCTION

Oral cavity is a strategic interface which provides easy access to the body [1] and saliva, equipped with components from both innate and adaptive immune systems, maintains an equilibrium within the oral biome.[2]

Quantity, pH, consistency, and composition dictate salivary contribution to oral health. Adequate salivary flow dilutes and buffers the acids where remineralisation tips the defensive scales towards strengthening the tooth surface in a favourable environment.[3] The Autonomous Nervous System (ANS) regulates salivary secretion and sympathetic influence of saliva is greatly enhanced by physical activity.[4] This study was undertaken to assess the influence of minimal physical activity on salivary parameters in children.

II. SUBJECTS AND METHODS

Fourteen children aged between the age of 5 to 11 years, visiting the Out-Patient Department of Pedodontics and Preventive Dentistry, without any prior dental treatment were selected for the study. Children with history of previous dental treatment, severely compromising pre-existing medical conditions, and children on long-term medication were excluded.[5]

Saliva was collected between 10 a.m. and 12 a.m. to rule out any circadian variations.[4] Children were advised not to eat/drink/perform rigorous exercise for at least 1-2 hours before collecting saliva. Height and weight of the child were recorded and ten minutes prior to the test the child was seated at rest in a chair close to the test area and was not encouraged to do any warm-up.[5] Oxygen saturation (SpO_2) and heart rate (HR) were measured using a finger pulse-oximeter. After rinsing the mouth with water, the child was seated in Coachman's position and unstimulated saliva was collected into a labelled, sterile plastic container by spitting method, for a period of 5 minutes at every 60 seconds.[6] Instructions were given to refrain from swallowing or any other

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minor oral movements during saliva collection.[7] Following this, the child was allowed to rest for 2 minutes and then asked to undertake the 6MWT.[5] The collected salivary sample was immediately assayed for pH using indicator strips (Qualigens, (pH 1 to 14) Thermo Fisher Scientific India Pvt., Ltd.) and salivary flow rate was evaluated by decanting the collected sample into calibrated test-tubes.[7]

Though 'maximum incremental cardiopulmonary exercise test' is considered to be the gold standard for evaluating an individual's response to aerobic exercise, a recent review of functional walking tests established the 6MWT to be advantageous in better reflecting activities of daily living because most of the routine day-to-day actions are performed at sub-maximal exertion levels. After giving instructions to the child, the 6MWT was conducted following the American Thoracic Society (ATS) guidelines on an enclosed area of flat, hard tiled 50m walkway. Orange traffic cones were used to designate the start point and the turn-around point along with bright coloured markings at every 3 feet (3 feet, 6 feet, 9 feet and 12 feet).[8] The children were asked to walk up and down the marked area to cover as much distance as possible in the allotted '6 minutes' time. The child was also instructed to stopand rest during the test if required.[5] This way, the total distance walked in the 6 minutes was recorded.

After the walk-test, SpO_2 and HR readings were noted again and the child was made to sit at rest for 5 minutes. A second sample of unstimulated saliva was collected in a manner similar to the first sample; pH and flow rate were evaluated immediately.

Within 30 minutes of collection, both pre-test and post-test samples of saliva were diluted at 1:1 with 9% NaCl, vortexed manually for 2 minutes and centrifuged (Remi Medico C-854/6) at 5000 rpm for 10 minutes. The supernatant was transferred to a labelled sterile aliquot and the sediment was discarded. All the aliquots thus prepared were then stored at -20^0C until they were assayed for amylase.[7]

The stored saliva samples were thawed to room temperature[7] and made up to 1:20 dilution using 0.9% NaCl and 0.5µL of reagent was then added to it (AUTOSPAN® Liquid Gold α-AMYLASE, $CNPG_3$ – Kinetic Assay, Amylase Mono Reagent 5ml). This mixture was vortexed manually for 1 minute and analyzed using a semi-automatic immunoassay analyser (Merilyzer – CliniQuant – micro). α-Amylase from the salivary sample catalyses the hydrolysis of 2-chloro-p-nitrophenol salt (CNP-Gal-G2) to chloronitrophenol (CNP). The rate of increase in absorbance due to formation of CNP is measured at 405nm in the analyser and is proportional to the α-amylase activity in the given sample. The results were noted at the end of 180 seconds with a delay time of 60 seconds.

$$\text{CNP - Gal - G2} + H_2O \xrightarrow{\alpha\text{-Amylase}} \text{CNP} + \text{Gal - G2}$$

Conducting the 6MWT, collection, processing, storage and amylase evaluation of all the salivary samples was done by a single operator under identical conditions and in a consistent method thorough out the study.

III. RESULTS

The results of the 6MWT are represented in Table 1 and the comparative evaluation of pre-test and post-test values of the measured salivary parameters are represented in Table 2. Salivary flow rate had an initial mean of 0.28±0.05 ml/min and a post-test mean of 0.41±0.11 ml/min, (Figure 1) showing an increase of about 46%. Salivary amylase changed from an initial 32.89±17.31mU/ml to 62.43±21.48 mU/ml (Figure 2) after the test, an increase of almost 89%.pH altered from an initial 6.97±0.15 to 7.24±0.16 (Figure 3) after the test, showing an adjustment towards alkalinity.The mean distance walked by the children was 1200.79 ft., ranging from a minimum of 930 ft. to a maximum of 1602 ft. (Table 1 and Table 2).

IV. DISCUSSION

According to the World Health Organisation (WHO) physical activity is defined as "a bodily movement produced by skeletal muscles that substantially elevates energy expenditure," and regular expenditure of energy by children aids in preventing coronary disease, diabetes and obesity (WHO).[9] Physical exercise also shows significant changes in salivary secretion exerted via sympathetic influence.[4][6] A study by Gulati A et al., in 2014 showed a prevalence of 21% inactivity in Indian children aged 3 to 11 years.[9] The Indian Report Card on Physical Activity For Children and Youth 2018, also puts the overall physical activity at 'Grade D' implying that only 25% of the children and youth practice physical activity for more than 60 minutes per day.[10] Routine activities during our daily life are performed at sub-maximal exertion levels and hence 6MWT was chosen in this study as an indicator of functional capacity.[11]

Salivary flow, ample in quality and quantity, is pertinent for maintenance of optimal oral hygiene.[2] Salivary glands produce about 0.5 to 1.0 L of saliva per day in response to neurotransmitter stimuli. This volume constitutes both stimulated and unstimulated saliva. Unstimulated salivary flow during the resting phases not only moistens and lubricates the mucous membrane, but is also an essential component of defensive mechanism in the oral cavity.[12] So, unstimulated saliva is primarily responsible for maintaining veracity of the oral tissues, whereas stimulated saliva takes care of digestion of food.[2]

Saliva forms a pellicle (a lubricating protein film) on enamel that inhibits acid attack on the tooth surface and prevents erosion of enamel.[7]pH and salivary flow rate are the physicochemical properties which play an important role in development of carious lesions.Increased salivary flow rate leads to enhanced clearance and concomitant reduction in microbial attack.[12] Apart from the IgA derived from lymphocytes, other non-immunologic factors such as amylase aid in bacterial clearance by selectively binding to some of the bacteria present in the oral cavity (ScannapiecoFA1994)[13]. Additionally, the key defensive properties of saliva with respect to tooth minerals (salivary buffering capacity, salivary clearance and degree of salivary saturation) are enhanced with increased salivary stimulation.[2]

Salivary flow is a non-immunological defense mechanism of saliva.[1] Clearance of food components and microorganisms from oral cavity into the stomach is one of the chief functions of saliva. A well-balanced interaction between the endogenous-exogenous microbial attack in the oral cavity and the host defense mechanisms mandates a sufficient amount of salivary volume to flush-out noxious as well as commensal microbes from the mouth. Based on the assumption that unstimulated salivary flow rate was an important factor for clearance (Dawes C 1983)[14]noted that rapid clearance is obtained due to high salivary flow rate as opposed to slower clearance from low salivary flow rate.[2] The normal unstimulated salivary flow rate is approximately 0.3 to 0.4 ml/min (Dawes C2008).[15]

In the present study, salivary flow rate enhanced from a pre-test mean of 0.28±0.05 ml/min to a post-test mean of 0.41±0.11 ml/min with a p value of 0.004 (Figure 1) which is statistically significant.

Ligtenberg AJ, Brand HS, van den Keijbus PA, Veerman EC (2015) also found an increase in salivary secretion rate from 0.62±0.24 ml/min to 0.78 ± 0.38 ml/min (25% up-regulation) following 10 minutes of moderate exercise and to 0.94±0.75 ml/min (51% up-regulation) following 10 minutes of high intensity exercise during their study.[4]

An increase in salivary flow rate enhances availability of organic and inorganic components of saliva. This up-surged flow rate aids in removal of remnant particulate food (due to its rinsing action), enhances clearance of acids and sugars from the tooth surface by hastening dilution and neutralising acids which dictate the initial onset and further progression of carious lesions. Such favourable conditions augment the anti-microbial protein output, leading to effective bacterial clearance.[2][7][16] Furthermore, an increase in flow rate is vital to the defensive mechanism of saliva because it acts as a means of transference of buffering agents, antimicrobial agents and also the mineral components thereby aiding in maintaining equilibrium between tooth structure demineralization v/s remineralization.[17] Additionally, the increased salivary flow rate is indispensable for acid dilution, flushing out of motile bacteria, clearance of particulate food entrenched in and around the teeth and removal of refined carbohydrates(Edgar M, Dawes C, O'Mullane D. Saliva l., 2004).[18]Caries risk increases exponentially with a low salivary flow rate whereas an increase in salivary flow rate assists caries prevention. Therefore, stimulating salivary flow rate is beneficial for oral health.[2][7][16]

The final composition of saliva arising from the major salivary glands depends strongly on the flow rate. The end product secreted into the mouth is always hypotonic. Based on the flow rate, whole saliva contains about 3-6 times less electrolytes than plasma.[19] Apart from water and ions, saliva has a complex mixture of close to a thousand proteins derived from various sources. Acinar cells are responsible for the production and secretion of high-abundant proteins like amylase, cystatins, gustin, while ductal cells produce growth factors and kallikreins.[7]The various proteins and the smaller organic proteins of the saliva protect the soft as well as the hard tissues of the oral cavity from harmful effects of pathogenic bacteria, dryness, frictional wear and erosion. Most of the salivary proteins are glycoproteins with a protein core and variable amounts of carbohydrates. Among all the total salivary gland-produced proteins, α-amylase is an important salivary enzyme.[2] Produced locally by salivary glands in the oral cavity, it is one of the larger digestive enzyme molecules found in saliva, and has a

molecular weight of around 55-60 kDa.[19]Parotid secretions are the main source of salivary amylase (80% of total secretion), whereas the sub-lingual gland secretions are primarily mucin-rich and comprise smaller amounts of amylase.[7] Breakdown of ingested starch to simple hexoses occurs in two phases beginning in the oral cavity with the hydrolysis of α–1, 4 glycosidic linkages of starch molecules by α–amylase.[19]It helps to split the dietary starch into maltose, maltotriose and dextrins.This heralds the digestion of food. But salivary α–amylases are active above a pH of 6 and inactivated in the gastrointestinal acidic environment. So, its activity is confined to the oral cavity.[2][7]

Under normal conditions, amylase is present in high concentrations with a primary function of digesting the macromolecules (starch and carbohydrates) and a secondary role of preventing bacterial attachment to oral surface and clearance of bacteria from the oral cavity (Marcotte H, Lavoie MC, 1998).[19]Dietary variations like calorie intake, the bacterial load, presence of periodontal conditions and carious process may be affecting the individual disparities in α–amylase levels. It increases in response to stimulation of the beta-adrenergic agonists independent of salivary flow.[19] The effect of physical exercise on salivary α–amylase levels has been demonstrated by Chatterton Jr RT, Vogelsong KM, Lu YC, Ellman AB, Hudgens GA (1996).[20]

In the present study, pre-test mean amylase of 32.89±17.31 mU/ml increased to post-test mean of 62.43±21.48 mU/ml (Figure 2). This is approximately 90% enhancement and could be attributed to 6MWT.

Amylase inhibits attachment of bacteria to the oral surfaces (Scannapieco FA 1994) [13] and therefore enhancement in salivary amylase levels after exercise might supplement the protective actions of saliva.[26] The increase in amylase levels is in agreement with other studies conducted. A study by Walsh NP (1999) demonstrated the α–Amylase levels to increase from a pre-exercise mean of 188±62 U/ml to a 5 post-exercise mean of 1085±384 U/ml, a 5-fold increase.[21] Khozaymeh F, Karimian J, Alikhani M, (2012)conducted a study that showed that α–Amylase levels changed from a pre-exercise mean of 59.57IU/ml to a post-exercise mean of 107.52 IU/ml during an aerobic endurance exercise activity.[22]

Microbial adhesion to the salivary pellicle is a complex process. Though several studies established that amylase had surface binding affinity to several microbes, especially species of Streptococci Douglas CW found that the adherence ability to sHAP and binding ability to amylase of a streptococcal strain had no correlation.[23] Also, Ligtenberg AJ, Walgreen-Weterings E, Veerman EC, De Soet JJ, De Graff J, and Amerongen AV (1992) reported hardly any adhesion of S.gordonii HG 222 to microtiter wells promoted by amylase.[24]The enzymatic cleft of amylase is the site where maltotriose (a trisaccharide with α-1,4 glycosidic bonds) binds and might lead to conformational changes of the enzyme on the surface of the hydroxyapatite. Therefore, in the current study, the enhancement of the α–amylase concomitant with an increase in the salivary flow rate leads to better enhancement of the collective properties of the salivary components in not only neutralising and buffering the acids, but also additionally benefits from the increased salivary flow rate resulting in better clearance.

pH is defined as "the negative logarithm of hydrogen ion concentration," and it refers to the degree of acidity or alkalinity of any given solution.[17] Mean pH of unstimulated saliva under normal conditions is 6.75 to 7.25.[1]

In the present study, pH showed a shift (Figure 3) from a pre-test mean of 6.97 ± 0.15 to a post-test mean of 7.24 ± 0.16 (p value 0.004 – Table 2) which is statistically significant.

Salivary pH varies with salivary flow rate and this pH enhancement leads to alteration in quantities of 4 phosphate ions, chiefly the tertiary ion $(PO_4)^{-3}$ which has an essential role in turning the tables from demineralization to remineralization of the tooth enamel.[17]

Previous studies (Pedersen BK (1991) [25] and Shepard RJ, Rhind S, Shek PN,(1994)[26] have shown that the immune functioning is intermittently suppressed following high-intensity exercise and therefore could act as a deterrent, leading to greater susceptibility to upper respiratory tract infections (Nieman DC, Nehlsen-Cannarella SL (1991).[27]But, in the current study, all the measured parameters demonstrated a positive shift in the defensive properties of saliva and hence, performing physical activities for a short period of time even at a sub-maximal level proves to be beneficial towards the goal of optimal oral well-being.

Though this is a pioneer effort trying to establish the effect of sub-maximal physical exercise on salivary components with an aim to ascertain the positive effects of the salivary defense mechanisms, a larger sample size and inclusion of children with challenged ambulation will aid in extrapolating the findings of this study. Such assessments need to be made in children with

challenged ambulation also to be able to ascertain a similar effect in them too.

V. CONCLUSION

Inactivity in children is deleterious in several ways and submaximal physical activity has been found to result in enhancement of salivary parameters in short bouts. Such repeated, smaller increments of short-time exercise episodes improve oral health, and therefore, this 6MWT could be very beneficial to children with challenged ambulation in augmenting their oral health.

KEY MESSAGE

Short episodes of sub-maximal physical activity may be beneficial in children in not only attaining good general health but also to achieve and maintain optimal oral health.

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Table 1: Results of the 6MWT in the sample population.

	N	Mean	Std. Deviation	Range	Minimum	Maximum
Age	14	8.86	1.70	6	5	11
Ht (in meters)	14	1.30	0.07	0.250	1.120	1.370
Wt (in Kgs)	14	24.76	4.06	14.0	19.3	33.3
BMI	14	14.58	1.76	6.92	11.77	18.69
Distance Walked (in feet)	14	1200.79	176.98	672	930	1602
HR – Pre (beats/min)	14	80.79	10.71	39	67	106
HR – Post (beats/min)	14	86.57	9.69	34	74	108
SpO_2 – Pre	14	98.79	0.58	2	97	99
SpO_2 – Post	14	97.36	1.60	5	94	99
Sal pH – Pre	14	6.97	0.15	0.4	6.8	7.2
Sal pH – Post	14	7.24	0.16	0.4	7.0	7.4
SFR – Pre (ml/min)	14	0.28	0.05	0.16	0.20	0.36
SFR – Post (ml/min)	14	0.41	0.11	0.32	0.28	0.60
α-Amylase – Pre (mU/ml)	14	32.89	17.31	62.24	7.34	69.58
α-Amylase – Post (mU/ml)	14	62.43	21.48	77.96	21.53	99.49

Ht – Height; Wt – Weight; BMI – Basal Metabolic Rate; HR – Heart Rate; SpO_2 – Oxygen Saturation; Sal pH – Salivary pH; SFR – Salivary Flow Rate.

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Table 2: Comparative evaluation of pre- and post-test values of salivary pH, flow rate and α–amylase represented as mean±SD.

	Mean ± SD		Pairedt-test		Correlation of Pre & Post-test	
	Pre(N=14)	Post (N=14)	t Value	P Value	r Value	P Value
Sal pH	6.97±0.15	7.24±0.16	-10.212	0.000	0.801	0.001
SFR (ml/min)	0.28±0.05	0.41±0.11	-6.209	0.000	0.717	0.004
α–Amylase (mU/ml)	32.89±17.31	62.43±21.48	-5.082	0.000	0.387	0.171

Sal pH – Salivary pH; SFR – Salivary Flow Rate.

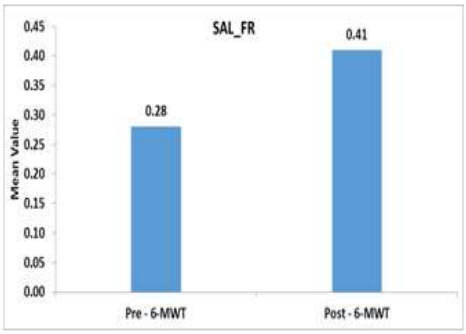

Fig1: Mean SFR values before and after 6MWT

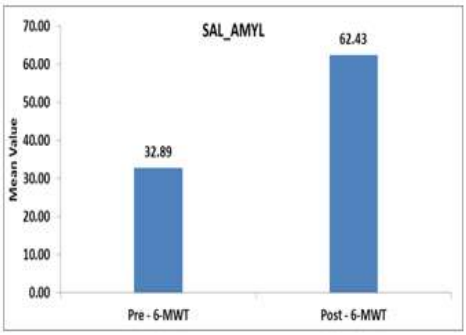

Fig 2: Mean levels of α-amylase before and after 6MWT

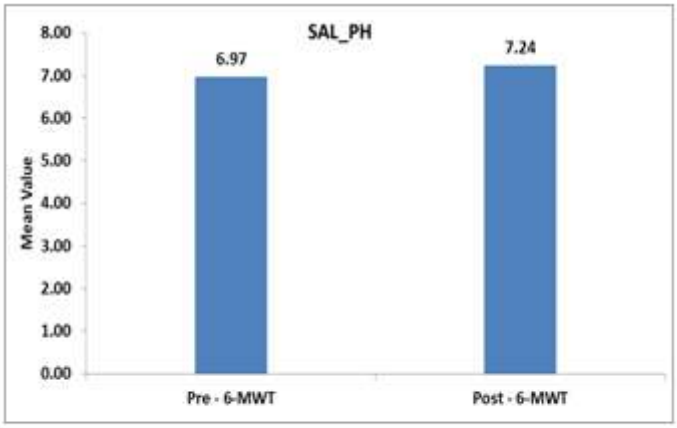

Fig 3: Mean salivary pH before and after 6MWT