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Impact of Fixed Orthodontic Therapy on Salivary Characteristics in Relation to Weight Status

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The aim of the present study is to evaluate the early impact of fixed orthodontic therapy on some salivary physical and chemical characteristics of patients in relation to their weight status. The sample consisted of 54 patients (25 males and 29 females; age between 16-18 years old) going under the course of fixed orthodontic treatment. Patients were categorized according to their Body Mass Index (BMI) into 3 weight status groups (underweight, normal weight, overweight and obese), then unstimulated salivary samples were collected from each patient who met specific inclusion and exclusion criteria during three time intervals; before bonding (1st visit), 2 weeks after bonding (2nd visit), and 4 weeks after bonding (3rd visit), then salivary samples are examined to evaluate some salivary physical properties (flow rate, pH) and some chemical properties (salivary calcium concentration, salivary phosphate concentration). A significant increase in salivary flow rate for all weight status categories during the 2ndvisit. A significant decrease in salivary pH for both underweight and overweight groups during 2nd visit with no significant change for normal weight group. A significant decrease in salivary calcium concentration for all weight status groups. And a significant increase in salivary phosphate concentration for all weight status groups. Exposure to fixed orthodontic appliances causes significant changes in salivary physical and chemical characteristics in early stages of treatment. These changes differ among patients with different BMI weight status, but with lesser intensity on normal weight patients than underweight and overweight and obese patients.

Keywords: Effect, orthodontic treatment, saliva, weight status.

The oral cavity can be described as a vastly complex environment, where numerous synergistic and antagonistic interactions take place at different times among its elements [1]. The kingpin in this environment is saliva, secreted by glands, which formed by water (99%) and other compounds (1%): Glucose, electrolytes (chloride ions, bicarbonates, phosphates, sodium ions, magnesium ions and calcium ions), nitrogen compounds (ammonia, urea) and proteins: enzymes, immunoglobulines, mucosal glycoproteins, albumins, oligopeptides, polypeptides and aquaporins and any additional constituent that may enhance the complexity of the oral environment in a variety of ways [2].

Fixed orthodontic appliances introduce an additional constituent to the oral cavity complex that may enhance environment in a variety of ways [3]. On the other hand, Orthodontic treatment leads

to changes in the oral environmental factors that encourage changes in salivary flow rate, viscosity, pH, bacterial count, increased plaque index, and other more things which all are factors supplement the risk of caries activity and shake the stability of the oral environment [4, 5, 6].

Associations between overweight and obesity with oral diseases is still swinging between significance [7, 8] and non significance [9], but generally still no establishment for direct causation [10]. So only what can be stated with some confidence is that obesity is one factor in a complex relationship between a multiple conditions, and this relationship requires further investigation. The same uncertainty and vague also surrounds the association between underweight status and oral diseases [11].

The aim of this study was to examine possible changes in some salivary physical and chemical characteristics (Flow rate, pH, and Calcium and Phosphate concentration) in subjects exposed to fixed orthodontic appliance, to determine if these changes differ in accordance to subjects' weight status. The hypothesis was that patients with normal weight would suffer less changes in there salivary characteristics than underweight and overweight and obese patients after the sudden impact of fixed orthodontic appliance.

MATERIALS AND METHODS

Study group

This was a prospective, self-controlled study. The study group consisted of fifty four patients (25 males and 29 females) were undergoing orthodontic treatment with fixed appliances in the Department of Orthodontics inside the specialized dental center for prosthodontics and orthodontics in Bab-Almoadham in Baghdad/Iraq. The inclusion criteria was only being inside the age range of 16-18 years old, while the exclusion criteria were that patients must **not** (1) had any systemic or oral diseases and allergies, (2) undergone orthodontic treatment before, (3) had congenital craniofacial anomalies, (4) had any oral or general pain still on going (5) been, or willing to be on a diet (fasting also), or on extreme dietary habits, (6) be treated with any space gaining appliance, (7) receiving any medications, (8) been a smoker or having any other addiction. Any patient who broke the previous criteria during the visits were also excluded, add to that the exclusion of patients if they didn't show for 2nd visit after 14 days, and 3rd visit after 28 days (±1 day). To minimize the number of unknown variables the same patients before commencing orthodontic treatment, were used as controls. Informed consent was obtained from all participants and their parents after a detailed explanation of the study. Ethical approval was obtained from the scientific committee of Department of Pedodontics and Preventive Dentistry, University of Baghdad.

This study was observational. Due to the dental center policy, all Patients must have received full dental treatment, plus a complete scaling and polishing and oral hygiene instructions prior to commence with orthodontic treatment. All patients were orthodontically treated using the same equipments.

At the beginning of each of the three visits; a data sheet were fulled for each subject; including records of height and weight taken for each patient in order to calculate their Body Mass Index (BMI) [12], next, according to the results of those calculations, patients were grouped into three categories (underweight, normal weight, overweight and obese); according to a standerd charts set by world health organization (WHO) in 2007; specific for each age (in months) and gender [13].

Salivary sample collection

A routine oral examination was done at the beginning of each visit. According to the standardizations set by Tenovuo and Lagerlöf in 1994 [14], each patient was asked to swash three times with water in order to remove any food remnants from the oral cavity, then having information about how to give their salivary sample by the use of draining method, before starting the procedure the patient was asked to spit for about a minute, collection started by asking the patient to sit quietly with their head bent down and their mouth open to allow the saliva to drip passively from the lower lip into a sterile plastic disposable jar, this method allow no stimulation and is more reliable [15].Collection of unstimulated saliva samples would start only between 9-11 am(in order to standardize the circadian rhythm). The collection of saliva continues for 10 minutes, in order to have an enough sample for running all

physical and chemical salivary tests (which must be over 3ml). Salivary samples were collected at three time points: before the beginning of orthodontic treatment (1st visit; baseline), after 14 days (2nd visit), and after 28 days (3rd visit) (±1 day). Collected saliva samples were immediately poured in a measuring cylinder to determine its volume, then amount needed for running chemical analysis was separated in a sterile disposable plain tube; in order to be centrifuged at 10,000 rpm for 5 minutes, after that the supernatant fluid was kept frozen (-20 °C) until analysis, while the rest of the sample were kept in a cooling box; for further physical analysis.

Protocol of Testing and Analysis

Salivary flow rate was immediately calculated through dividing the volume of the collected salivary sample (in milliliters) by the duration used for collecting the salivary sample (in minutes) [16]. The duration was used for collection was standardized as 10 minutes for all the study samples.

Salivary pH was determined by the use of a digital pH meter (Hanna instruments / USA), which was calibrated and adjusted at the beginning of every sampling day by the use of three known pH solutions (pH=3, pH=7, pH=9) which where freshly prepared according to the manufacturer instructions, then after calibration with each of the solution the meter was washed with running water (pH=7) to remove any remnants, then cleaned with alcohol and let to dry. The pH meter head was fully immersed inside the sample collection cup, let for 30 seconds inside to get a stable final reading , which was recorded on the patient‘s record sheet. After reading of each sample; the process of washing of the pH meter with distilled water and alcohol was repeated.

Analysis of salivary Calcium was done through the use of Calcium kit (Liquicolor kit / Human / Germany), colorimetric test for the quantitative determination of calcium total in human saliva was done. The reaction of Calcium ions with o-cresolphthalein-complexone in an alkaline medium will form a purple colored complex; the absorption of this complex is proportional to the calcium ion concentration in the sample [17, 18].

Analysis of salivary Phosphate was determined through the use of inorganic phosphorus kit (phosphorus liquirapid /Human / Germany), by UV test for quantitative determination. The Principle is that Phosphate ion reacts with molybdate within a strong acidic medium to form a complex; acid formation of a phosphomolybdic complex would then subsequently reduce in alkaline medium originating a blue molybdenum color. The intensity of the color in the near UV is directly proportional to the inorganic phosphorus concentration in the sample [19, 20].

Statistical Analysis

The study sample which include 54 subjects (>30 or 40) distributed normally, that's regardless of the underlying distribution and shape of the data in accordance to the central limit theorem and law of large numbers (18,19). Statistical analysis was performed through ANOVA test with the use of (Statistical Package for Social Science SPSS version 21), using General linear model for repeated measures. P value <0.05 was considered the limit of statistical significance.

RESULTS

Table 1 shows that In spite of changes in patients' Body Mass Index (BMI) through the three visits, but they were not significant through the three visits.

Each of Table 2, 3, 4 and 5 took one salivary physical or chemical characteristics readings data, and compare the readings of each weight status group with through visits, also compares the readings data between every two visits (1st x 2nd, 1st x 3rd, 2ndx 3rd).

Table 2 shows that for the salivary flow rate, there was a significant increase during the 2nd visit for all weight status groups, those high levels hold for underweight and overweight groups during the 3rd visit, while for the normal weight group the levels return to its primary levels. The highest significant change during the second visit was for underweight, followed by normal weight, next overweight.

Table 3 shows that for the salivary pH, there was a significant decrease during the 2nd visit for both underweight and overweight groups, those levels slightly goes up(but still under normal) during the 3rd visit in a non significant way, while normal weight group had a non significant changes during the 2nd and 3rd visits.

Table 4 shows that for salivary calcium

concentration levels, there was a non significant decrease in all three groups during 2nd visit; transforming into a significant decrease during the 3rd visit for all weight status groups.

Table 5 shows that for salivary phosphate concentration level, there were different non significant changes during the 2nd visit; followed by a significant increase for all weight status groups. In general there was a significant change in all physical and chemical characteristics for all weight status groups; except for pH level of normal weight group.

Table 1. Descriptive and statistical test of weight status change between visits by gender

Weight status	Gender	1st visit		2nd visit		3rd visit		ANOVA	
		Mean	±SD	Mean	±SD	Mean	±SD	F	Sig
Underweight	Male	18.34	0.26	18.14	0.28	18.27	0.13	0.860	0.439
	Female	17.17	1.49	17.05	1.47	17.15	1.50	1.002	0.381
Normal weight	Male	21.14	1.76	21.01	1.72	21.22	1.64	2.521	0.110
	Female	19.90	2.27	19.74	2.32	19.79	2.35	0.775	0.473
Overweight and obese	Male	30.82	4.33	30.48	4.01	30.02	3.28	2.668	0.150
	Female	27.54	5.04	27.36	4.64	27.54	5.36	0.050	0.880

Table 2. Descriptive and statistical test of salivary flow rate change through visits and by weight status

Measure		ANOVA repeat		3rd visit		2nd visit		1st visit		Weight status
P	visits	p	F	±SD	M	±SD	M	±SD	M	
0.009*	1st x 2nd	0.003*	9.048	0.15	0.82	0.15	0.85	0.15	0.67	Under-weight
0.000*	1st x 3rd									
1.000	2nd x 3rd									
0.019*	1st x 2nd	0.016*	4.607	0.23	0.68	0.24	0.75	0.24	0.64	Normal weight
1.000	1st x 3rd									
0.138	2nd x 3rd									
0.035*	1st x 2nd	0.023*	4.945	0.17	0.56	0.21	0.58	0.21	0.45	Over- weight& obese
0.025*	1st x 3rd									
1.000	2nd x 3rd									

* Significant ($p < 0.05$)

Table 3. Descriptive and statistical test of potential hydrogen (pH) change among visits and by weight status

Measure		ANOVA repeat		3rd visit		2nd visit		1st visit		Weight status
P	visits	p	F	±SD	M	±SD	M	±SD	M	
0.000*	1st x 2nd	0.000*	10.827	0.19	7.06	0.32	6.93	0.32	7.19	Under-weight
0.144	1st x 3rd									
0.100	2nd x 3rd									
0.902	1st x 2nd	0.148	2.027	0.29	6.91	0.28	6.94	0.32	7.00	Normal weight
0.120	1st x 3rd									
1.000	2nd x 3rd									
0.001*	1st x 2nd	0.001*	9.660	0.03	6.9	0.29	6.82	0.35	7.02	Over- weight& obese
0.097	1st x 3rd									
0.410	2nd x 3rd									

* Significant ($p < 0.05$)

Table 4. Descriptive and statistical test of salivary calcium concentration change through visits and by weight status

Measure P	visits	ANOVA repeat P	F	3rd visit ±SD	M	2nd visit ±SD	M	1st visit ±SD	M	Weight status
0.340	1st x 2nd	0.001*	13.141	0.423	4.213	0.424	4.8344	0.658	5.0281	Under-weight
0.005*	1st x 3rd									
0.003*	2nd x 3rd									
1.000	1st x 2nd	0.003*	8.664	0.509	4.468	0.471	4.7500	0.550	4.7940	Normal weight
0.010*	1st x 3rd									
0.020*	2nd x 3rd									
1.000	1st x 2nd	0.006*	10.081	0.235	4.231	0.425	4.6454	0.452	4.6500	Over- weight& obese
0.024*	1st x 3rd									
0.020*	2nd x 3rd									

Table 5. Descriptive and statistical test of salivary phosphate concentration change through visits and by weight status

Measure P	visits	ANOVA repeat P	F	3rd visit ±SD	M	2nd visit ±SD	M	1st visit ±SD	M	Weight status
0.276	1st x 2nd	0.003*	11.676	1.864	5.05	.8797	3.625	0.853	3.74	Under-weight
0.017*	1st x 3rd									
0.007*	2nd x 3rd									
0.847	1st x 2nd	0.001*	10.864	2.010	4.784	1.046	3.504	1.105	3.70	Normal weight
0.015*	1st x 3rd									
0.002*	2nd x 3rd									
0.731	1st x 2nd	0.000*	21.976	1.872	6.307	1.286	4.269	1.15	3.98	Over- weight& obese
0.000*	1st x 3rd									
0.002*	2nd x 3rd									

* Significant ($p < 0.05$)

DISCUSSION

Association between weight status and oral and dental health is still not fully proved or understood and needs more research to figure more relations of the causes‘ combination [23]. Fixed orthodontic appliances deleterious influence on oral health and environment through affecting saliva is well documented [4, 5]. Will normal weight subjects withstand the harmful change in salivary characteristics more than underweight, overweight and obese patients? This study was designed to evaluate this hypothesis.

To minimize the influence of variables, a prospective, self-controlled study has been designed with the same subjects before orthodontic treatment to be used as controls. Many previous studies used a similar design [24], while others used independent control groups [25]. This study considered different than other studies because of categorizing early effect of fixed orthodontic treatment according to weight status of the patients, while previous studies concentrated on the general effect of fixed orthodontic appliance on salivary characteristics. Also what increases its distinct is it Checks with patients after two weeks and then after 4 week from the first visit, which is very important; to determine the true impact of orthodontic appliance away from human body adaptation. Unstimulated whole saliva mimics basal salivary flow rate and is the secretion that provides protection to oral tissues and is present for about 14 hours a day [26].that’s the reason why this study considered unstimulated saliva in its analysis.

The earliest significant impacts of fixed orthodontic appliance appeared to be on the salivary physical characteristics (Flow rate, pH) during the first two weeks (2nd visit), while chemical changes(calcium and phosphate concentration change) took more duration to show significant changes; that appeared after 4 weeks(3rd visit). The general increase in flow rate for all weight status categories is similar to many studies [5, 27]. The sudden increase during the first 2 weeks may be referred to combination of many factors that may lead to increase flow rate; including pain [28], increase diet with watery components [29], or a normal physiologic response to presentation of a foreign body inside the oral cavity [30]. The graduation of significance levels of change between 1st and 2nd visits (in table 2) from underweight (p=0.009), to normal weight (p=0.019), then over weight (p=0.035) gives pain the upper hand in causing increase of flow rate; that's when know that pain threshold is higher for overweight and obese people than lesser weight [31]; means that overweight persons have a lesser pain sensation than people with normal weight or underweight, which is consecutive with the results, while the steady continuous increase during the 3rd visit may be related to the physiologic responses only.

The data of the present study showed that there was a decrease in salivary pH, which agreed with other studies [26, 32]. This decrease was significant for underweight and overweight group during the 2nd visit; and then both groups record a non significant increase that still less than the initial value before beginning with orthodontic treatment, While for the normal weight group the decrease was non significant during 2nd and 3rd visits. similar re-arise of pH values after one month of treatment is recorded by other studies [27], one of the main causes of decreasing pH is the increase of acidogenic and aciduric bacteria due to presence of braces and difficulty of maintain good oral health [5, 6] , and that may lead to the fact that the oral flora and homeostasis of normal weight subjects is stronger and less prone to change than both underweight and overweight and obese subjects, this can be proved by the fact of higher caries rate in them than in normal weight subjects [33],caries, the Multifactorial disease that drop in pH is the main event in initiating it [34].

The change in chemical characteristics seems to need more time to be significant, because it only shows that at the 3rd visit, also these changes showed no significant difference between weight status groups, may be because of the short period of following up the patients (only one month); and the differences will be more obvious in more time of exposure to the fixed orthodontic braces, or it may be not related to weight status of the patients, although taking a second look on tables 4 and 5 can notice that the significance levels of normal weight group and overweight group are kind of similar in changes between 1st to 2nd visit; and less in change significance than underweight group , this may turn the looks toward a diet and micronutrients reservoir to compensate dietary defects during orthodontic treatment [28] that the normal and overweight persons might had but lacked in underweight persons. Generally, the decrease in salivary calcium is similar to that recorded by Teixira *et al* (2012) [26], decreased salivary pH will promote demineralization, but the increased effect of washing away due to the increased flow rate; plus abundance of dental plaque which act as a reservoir of calcium lead to decrease salivary calcium concentration. phosphate concentration is found to be increased in this study, resembling study by Gandhy and Damle in 2003 [35],which give an explanation through stating that Phosphate ions serve to maintain salivary pH and these ions are present in the form of PO4 3- ions. So when the pH becomes highly acidic this ion gets converted into HPO4 2- and then H2PO4- thus it acts as one of many salivary buffers, So saliva is not saturated with phosphate, chances for enamel dissolution and release of phosphate ion increases.

CONCLUSIONS

Exposure to fixed orthodontic appliance causes significant changes in both salivary physical (flow rate, pH) and chemical (calcium and phosphate concentrations) characteristics. Normal weight subjects can withstand direct and sudden impact of fixed orthodontic appliance concerning salivary physical characteristics more than underweight and overweight and obese subjects, while no difference sensed among weight statuses concerning changes in chemical characteristics.

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