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Relationship between Growth Hormone Level and Growth Parameters among Primary School Students with Malocclusion in Center of the Middle city of Iraq

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Abstract

Background: Growth hormone (GH) is a critical regu-lator of the growth process in children. Malocclusion is a dento-skeletal disorder that may affect the function and aesthetics of patients and thereby cause impairments in their quality of life and social interactions. The relation-ship among malocclusion, body mass index (BMI), and GH level in children is debatable. Aim of the study: This study aimed to assess the relation of GH levels to mal-occlusion and growth parameters among a group of primary school students. Subjects and Methods: A cross-sectional comparative study was performed among school children aged 10–11 years in the center of Kerbala City, Iraq. Only 88 students were randomly selected to test the relationship between GH and BMI. ELISA was applied to examine salivary samples to estimate the GH levels. The presence of malocclusion was detected through intraoral examination in accordance with angle classification. Overjet and overbite were measured by the dental caliper Vernier. The growth parameters for BMI (height and weight) were analyzed. Results: This study showed a high prevalence of overweight females over males. No relationship was found between demographic variables and BMI. Low levels of GH were reported in overweight patients. Class I malocclusion was higher than class II in all BMI categories. Increased overjet and overbite were mostly higher than normal in the BMI categories. The presence of posterior crossbite was higher in normal BMI, whereas obesity was the least. All these results showed no significant association between overbite and BMI. Conclusion: An inversely propor-tional relationship exists between basal metabolic rate and GH levels among school children.

Keywords: *salivary growth hormone; BMI, school children; malocclusion.*

Introduction

Occlusion is described as any contact between opposing dental arches' teeth. A mix of hereditary and environmental factors mainly causes malocclusion[26.]. Previous research demonstrated that it can lead to tooth decay ,masticatory and phonetic problems, mouth breathing, , gingival disease, jaw joint problems, and impaired aesthetics[(23,24)].

Body composition refers to the relative amounts of lean and fat tissue in the body. Lean body mass is the body's total amount of lean/fat-free tissue (muscles, bones, skin, organs, and body fluids). By contrast, fat mass is the body mass of fat tissue, an important

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indicator of general health. Body mass index (BMI) is a statistical measure of an individual's weight measured in accordance with height [36].
The growth process can be defined as an increase in body mass, which results in a gain in size. Growth is a complicated process primarily affected by environmental, genetic, and hormonal factors. It is a continual process that includes changes in stature, proportion, and body composition [(30,37)].

Growth hormone (GH) is a peptide hormone that stimulates growth, cell reproduction, and regeneration in humans . Secreting is usually increasing during childhood reaching a peak level in puberty[15].

It is essential for human growth[20]. Researchers showed that GH's effect on body tissues can be described as anabolic (building up). The effect of GH causes an increase in height during childhood and adolescence. It can also promote lipolysis; and stimulate the immune system[11].
Salivary diagnosis is an increasingly exciting field in dentistry, internal medicine, endocrinology, pediatrics, immunology, clinical pathology, psychology, and sports medicine[14]**.** A growing number of drugs, hormones, and antibodies can be monitored in saliva, which is an easily obtainable, non-invasive diagnostic [(18,35)]**.**
Research question: Is there a relation between GH with malocclusion and BMI?
Null hypotheses: Malocclusion is unrelated to BMI, GH, and dental malocclusion.
Aim of study:
This study was conducted among primary school children to assess the following:

- GH levels in students
- Relation of BMI to malocclusion

Materials and Methods

This study was approved by the Research Ethics Committee of the College of Dentistry, University of Baghdad in Iraq (approval number: no572, date: 17-4-2022). It was conducted among primary school pupils aged 10–11 years old in the fourth and fifth grades in the center of Karbala City, Iraq. Legal permission was obtained from the Ministry of Education/Karbala City to perform the clinical examination. Questionnaire survey was conducted in primary schools to ensure the cooperation of staff and teachers.

The total sample size was 400 students. Eighty-five students were randomly selected among the entire sample to assess the GH level and BMI as growth parameters in malocclusion. The enrolled patients were Arabian Iraqis in origin. Both genders were included. The patients' selection was based on the presence of the four upper and lower incisors and the first four permanent molars that fully erupted. The enrolled samples were free of syndromic and cleft lip/ palate, and they had no history of orthodontic treatment.

- Assessment of GH:

A total of 85 patients were randomly selected among the study groups for measurement of salivary GH in feeding pattern.

- Patient preparation before salivary collation

Unstimulated saliva from a internally displaced person was collected by passively drooling into the collection tube with the use of a 5-minute timer in accordance with the University of Southern California School of Density guidelines for saliva collection[23]**.** Students were instructed not to eat nor drink for at least 1 hour before the test session. Smoking, drinking coffee, and chewing gum were prohibited throughout this hour.Students were advised to wash their mouth with deionized or distilled water several times and then remain calm or relaxed for 5 minutes. Next, they were instructed to minimize their movement, especially their mouth, during collection. They were also

instructed to slope the head forward, keeping the mouth slightly open to allow saliva to drool into the collection tube.

- ELISA kit materials and instruments

The following instruments and materials were prepared to perform ELISA: microwell coated with hGH mAb, microliter well reader capable of reading absorbance at 450 nm, disposable pipette tips, graph paper, absorbance paper, precision pipettes, distilled water, HGH standard: six vials (ready to use), HGh enzyme conjugate: one bottle (ready to use), TMB Substrate: one ready-to-use bottle, and 20* wash concentrate: one bottle.

- Saliva storage and preparation

The salivary samples were centrifuged for approximately 20 minutes at 2000–3000 RPM. Afterwards, they were stored at about −20 °C.

- Salivary GH analysis

Saliva storage and analysis were performed in accordance with the manufacturer's instruct tions. Before the assay, reagents were allowed to stand at room temperature. All reagents were gently mixed before use.

1. The desired number of coated strips at room temperature was placed.

2. hGh standards (50 *Ml)* were pipetted, and the patients' saliva was pipetted into strips.

3. High enzyme concatenate (100 ml) was added to all wells.

4. The plate was covered and incubated for 30 minutes at room temperature (18 °C–26 °C).

5. The liquid from all wells was removed. Then, the wells were washed three times with 300 *Ml* of wash buffer and bloated using an absorbent paper towel.

6. TMB substrate (300 *Ml*) was added to all wells.

7. All wells were incubated for 10 minutes at room temperature.

8. Stop solution (50 *Ml*) was added to all wells. The plate was shaken gently to mix the solution.

9. The absorbent was read by an ELISA reader at 450 *Ml* within 15 minutes after reading the stopping solution.

- Procedure

kit uses ELISA on the basis of biotin double-antibody sandwich technology to assay human GH. First, GH was added to the wells, which were pre-coated with GH monoclonal antibody and then incubated. Second, anti-GH antibodies labelled with biotin were added to unit with streptavidin–HRP, which forms an immune complex. Unbound enzymes were removed after incubation and washing. Substrates A and B were then added. The solution turned blue and changed into yellow with the effect of acid. The shades of the solution and the concentration of GH are positively correlated.

- Dental examination

Oral examination for malocclusion was performed while the child was seated on an ordinary school chair in an area with good daylight and without any artificial light source. During the assessment of the presence of overjet or overbite, Angle's classification was performed using dental mirrors, a millimeter-graded dental vernier, and cotton rolls. The patient was guided to close in centric occlusion, and the occlusion was evaluated. The obtained information and dental examination results were recorded in a designated case examination sheet.

- Anthropometric measurements

A digital weighing scale was used to measure children's weight in kilograms (Kg). The gear was adjusted at the beginning of every day[31]. Children's weight was measured with light clothes without head cover or shoes and without touching anything. A normal measuring tape was used to measure children's height to the nearest of 0.1 cm. The measurement was accomplished while the child was standing in an upright position following shoe removal, with feet parallel to each other [10].

- BMI

BMI was measured as weight/height^2 (kg/m^2). The data of weight and height were then changed into BMI for age z-scores by using the WHO AnthroPlus software that utilizes the growth reference of WHO (2007). Z-scores permit the assessment of an individual's BMI or weight and height after being modified for age and gender in relation to a reference population, conveyed in standard deviations (SDs) from the reference mean. The following cut-offs for BMI for age z-scores were utilized to classify the weight status of children in accordance with WHO (2007):

• Thinness [< −2 (SDS)]

• Normal weight (from ≥ −2 to ≤ 1 (SDS)]

• Overweight [from > 1 to ≤ 2 (SDS)]

• Obesity [> 2 (SDS)]

as: < 5% (underweight), 5%–85% (normal), 85%–95%, (overweight), and > 95% (obese).

Statistical analysis

Data description, analysis, and presentation were performed using SPSS (version 22, Chicago, Illinois, USA). Sta tistical analyses can be classified into two categories: descriptive and inferential analysis. Descriptive analysis included frequency, percentage, mean, and standard error calculation. Thus, the inferential analysis included independent sample T-test, paired T-test, Pearson correlation, Fisher, Chi-square and ANOVA. Simple and cluster graphs were constructed to represent the data**.** The probability of error (P-value) was established at 5%.

Results

A cross-sectional observational study was conducted in the period of February–June 2021.

1. Relationship between BMI and gender

Table 1 shows the relationship between BMI and gender among participants. The findings showed that in most BMI categories, females recorded higher percentages than males except for overweight value. No statistical significant association was found (*p*-value = 0.504).

Table 1: Demographic data of BMI according to gender

BMI	Males		Females		Total	
	No	**%**	**No**	**%**	**No**	**%**
Under	66	43.14	87	56.86	153	38.25
Normal	84	45.65	100	54.35	184	46.00
Over	23	50.00	23	50.00	46	11.50

Obese	5	29.41	12	70.59	17	4.25	

Chi square = 2.344, p value = 0.504 NS

2. Relationship between nutritional status and demographic factors

Table 2 shows the relationship between nutritional status and demographic factors among participants. The findings showed that no significant association between nutritional status and demographic variables except in mother's education. especially, underweight, overweight, and obese BMI were the highest in the college with significant results.

Table 2. Distribution of nutritional status indicators in relation to demographic factors

BMI		Under		Normal		Over		Obese		X^2	P value
		N	**%**	**N**	**%**	**N**	**%**	**N**	**%**		
CHR	**First child**	44	35.77	56	45.53	18	14.63	5	4.07	7.865	0.548
	Second child	48	37.21	58	44.96	14	10.85	9	6.98		
	Third child	30	41.10	32	43.84	9	12.33	2	2.74		
	Above	31	41.33	38	50.67	5	6.67	1	1.33		
FE	**Illiterate**	10	58.82	6	35.29	1	5.88	0	.00	18.212	0.208
	Primary	16	42.11	19	50.00	2	5.26	1	2.63		
	Intermediate	21	42.86	23	46.94	3	6.12	2	4.08		
	Sec.	37	43.02	42	48.84	6	6.98	1	1.16		
	College	54	34.18	68	43.04	27	17.09	9	5.70		
	Higher	15	28.85	26	50.00	7	13.46	4	7.69		
ME	**Illiterate**	16	64.00	9	36.00	0	.00	0	.00	31.531	**0.003**
	Primary	33	40.74	41	50.62	5	6.17	2	2.47		
	Intermediate	27	49.09	23	41.82	3	5.45	2	3.64		
	Sec.	23	31.08	42	56.76	7	9.46	2	2.70		
	College	43	32.58	53	40.15	25	18.94	11	8.33		
	Higher	11	33.33	16	48.48	6	18.18	0	.00		
MO	**Absence**	105	38.60	128	47.06	27	9.93	12	4.41	2.117	0.548
	Present	48	37.50	56	43.75	19	14.84	5	3.91		

CHR: Child Rank ,FE: Father Education ,ME: Mother education ,MO: Mother Occupation.

3. Relationship between BMI and malocclusion

CLI was higher than CLII in all BMI categories, mainly increasing for OJ and OB, and higher than normal in BMI categories. The presence of crossbite was found to be higher in normal BMI where as obesity was the least, but all these results showed no significant association between OB and BMI.

Table 3. Relationship between BMI and malocclusion (assessment of the relation between BMI and dental development).

	Occlusal status	Under		Normal		Over		Obese			
		No	**%**	**No**	**%**	**No**	**%**	**No**	**%**		
Molar Relation	**CL I**	112	36.84	140	46.05	38	12.50	14	4.61	2.135	0.545
	CL II	41	42.71	44	45.83	8	8.33	3	3.13		
Over Jet	**Normal**	50	31.65	79	50.00	23	14.56	6	3.80	6.135	0.105
	Increase	103	42.56	105	43.39	23	9.50	11	4.55		

Over Bite	**Normal**	66	35.68	85	45.95	29	15.68	5	2.70	7.754	0.051
	Increase	87	40.47	99	46.05	17	7.91	12	5.58		
Posterior Crossbite	**Presence**	17	40.48	21	50.00	3	7.14	1	2.38	0.946	0.820
	Absence	136	37.99	163	45.53	43	12.01	16	4.47		

Discussion

One of the critical factors affecting youngsters' growth and health is nutrition. Factors, such as eating habits, quality of food, and timing of meals, are essential to protect human bodies from malnutrition. Malnourished children are more prone to infections, systemic diseases[13], dental caries[2,16], enamel defects[39,6], malocclusion, disturbance of the growth cycle [1,14], and periodontal diseases[28].

In contrast to a previous study[21], the present study found a higher prevalence of overweight (increased BMI) in females than in males. Meanwhile,[32] significantly increased BMI scores were observed in females over males. The findings also reported no statistically significant difference between participating children's nutritional status and demographic factors. They coincided with[17], who reported a significant association between malocclusion and BMI.

GH is a critical regulator of the growth process in children[25] . Examining salivary samples to detect the level of GHs was a perfect method for children who could feel afraid of performing the conventional blood sampling method[11]. Its level is strongly associated with gender, because it was found that GH had higher levels in females. A study showed that the female body secretes more than male GH levels[4,38]. A higher prevalence of increased GH levels was associated with under weighed children with low BMI according to[22]. Malnourished children are more prone to malocclusion, disturbance of growth cycle[14,31] . This study was revealed that there were no statistical significant difference between BMI and gender (p-value= 0.504) of the different types of malocclusion. On the contrary with this esults[13] reported significant relationship between the development of malocclusion and under weighted children. It was also reported that there was no statistical significant difference between the nutritional status and demographic factors of participated children[13].

About result of relation between BMI and malocclusion there was non-significant difference between the malocclusion and BMI in the present study this supported by these studies and agree with who reported that there was inverse association between them. This come due to the limitations in the sample size, age and the region from which the sample had taken, the sample selected from only one city from Iraq not all or some of cities[19].

In conclusion, an inversely proportional relationship exists between basal metabolic rate and GH levels among school children.

Conclusion

This study reported an inversely proportional relationship between basal metabolic rate and GH levels among school children. It showed that class I malocclusion was higher than class II in all BMI categories. Increased overjet and overbite were mostly higher than normal in the BMI categories. The presence of posterior crossbite was found to be higher in normal BMI, whereas obesity was the lowest. All these results showed no significant association between overbite and BMI.

Conflict of interest: None

Author contributions

HAS and SST: study conception and design. HAS: data collection. HAS: methodology. HAS: statistical analysis and interpretation of results. HAS: preparation of original draft manuscript. HAS: writing - review and editing. SST and SR: supervision. All authors reviewed the results and approved the final version of the manuscript to be published.

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Informed consent

Informed consent was obtained from all individuals included in this study or their guardians.

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