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The Effect of Sleep Duration on Salivary Growth Hormone and Dental Occlusion in Relation to Height Status among Kindergarten Children

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Abstract: ***Background:*** ***Sleep means relaxation and rest, it is an essential biological process that is required on a daily basis for all humans regardless of sex, age or ethnic origin, sleep deprivation is becoming most common in today's society compared to a few decades ago, short sleep duration adversely impacts human health. The purpose of this study was to assess the effect of sleep duration on preschool children growth and their dental occlusion.*** ***Material and Methods:*** ***The sample composed of 150 children of 5 years old who were selected from different kindergartens in Baghdad. The children were divided into two groups according to sleep duration per day. For salivary Human Growth Hormone (HGH) identification, the unstimulated salivary sample was collected from 80 children under standardized condition then analyzed for assessment of HGH level. The occlusal assessment was carried out with the teeth in centric occlusion then height for age Z-score was calculated.*** ***Results:*** ***The results reported that the mean value of salivary HGH was lower among children with 7-9 sleeping hr./day than children with 10-12 sleeping hr./day with statistically highly significant difference (P< 0.01). Concerning each gender the same highly significant difference finding was recorded (P< 0.01). The relation between salivary HGH concentration and sleeping hr./day showed strong highly significant relation in positive direction for preschool children with 7-9 sleeping hr./day; for preschool children with 10-12 sleeping hr./day, and for total sample. The findings showed that 35.8% of preschool children with 7-9 sleeping hr./day had a distal step, followed by 12.6% and 1.3% preschool children had flash terminal plane and mesial step respectively, while 33.8% of preschool children with 10-12 sleeping hr./day had a mesial step, followed by 10.6% and 5.3% preschool children had flash terminal plane and distal step respectively. The results reported that the mean value of HAZ was highly significantly lower among children with 7-9 sleeping hr./day than children with 10-12 sleeping hr./day (P< 0.01). Concerning each gender the same finding was recorded with statistically highly significant difference (P< 0.01). For preschool children with 7-9 sleeping hr./day, the relation between HAZ and sleeping hr./day was weak non-significant in positive direction. For preschool children with 10-12 sleeping hr./day, the relation between HAZ and sleeping hr. /day was weak non- significant in negative direction. For total preschool children, the relation between HAZ and sleeping hr./day was highly significant in positive direction. The results also showed that the percentage of HAZ above average (above 1 SD), with average (0 SD) and below Average (below -1 SD) were 13.3%, 50.7% and 17.3 respectively, with 13.3% and 5.3% children were suffer from stunting and sever stunting (below -2 SD and -3 SD of the reference CDC population) respectively among preschool children with7-9 sleeping hr./day, while the percentage of HAZ above average (above 1 SD), with average (0 SD) and below average (below -1 SD) were 41.3%, 56% and 2.7% respectively, with no children were suffer from stunting or sever stunting among preschool children with 10-12 sleeping hr./day.*** ***Conclusions:*** ***The findings of the present study showed that the sleep duration per day might affect children growth and their dental occlusion.***

Keywords: Sleep, Growth Hormone, Height, Occlusion

1. Introduction

The most important period of human being development and growth are the first years of life [(1)]. Sleep; like diet and physical activity, plays an important role in the maturation, growth, and health of children and adolescents by allowing for the diurnal rhythm of hormones related to maturation, growth and energy homeostasis [(2)]. Sleep had received much less attention than physical activity and dietary intake [(3)].

Human growth hormone (HGH) is secreted from the anterior pituitary gland, GH is secreted in a pulsatile fashion, two of the biggest factors that play a role in the release of this hormone are sleep and exercise, human being spend approximately one-third of their lives sleeping [(4)]. The major secretary HGH pulse occurs just after sleep onset and continues to rise during the first 4 hours [(5)]. The growth of bodily tissues is the primary role of HGH and its highest peak at younger age while begins to slowly decrease as growing older, HGH is involved in the turnover of muscle tissue, as well as the remodeling of collagen tissues and bone [(6)].

The studies concerning the relationship between sleep and children growth are few. Kohyama et al. [(7)], showed that the average values for height and weight were not correlated with the nocturnal sleep onset time.

While Yokomaku et al. [(8)], showed that late night sleep, waking up late and irregular sleep pattern through the week had negative effects on children health ages 4 to 6 years old.

Firoozabady et al. [(9)], findings showed that there was a direct significant relation between sleep disorders and behavioral problems in primary school children. Moradnia et al [(10)], showed that children sleep habits does not affect their growth.

Occlusion in the primary dentition plays a significant role in determining the space for and occlusion in the succeeding permanent dentition, the characteristics of the permanent dentition occlusion can be predicted very well from these key features of occlusion during the formative years [(11)]. There was no previous Iraqi study concerning the relation between sleep duration and preschool children growth and their dental occlusion, so this study was designed to investigate these relationships.

2. Materials and Methods

The sample of this study involved one age 5 years old preschool children, devoid of any systemic disease, physical or mental abnormality. The sample size composed of 200 children who were collected from different kindergartens in Baghdad, 50 children were excluded who did not fulfill the criteria of this study; these criteria included:

- Complete primary dentition without any erupted permanent teeth
- Sound primary second molars.

The numbers of sleeping hours per day and birth date were recorded from child's parent by questioner, then it assessed according to sleep quid lines [12]. The children were divided into two groups according to sleep duration per day. For oral examination, the child asked to sit on an ordinary chair and examination done under daylight. For salivary Human Growth Hormone (HGH) identification, the unstimulated salivary sample was collected from 80 children under standardized condition that cited by Navazesh and Kumer [13], and immediately placed it in ice box until reach the laboratory; at the laboratory, each salivary sample was centrifuged according to bioactive diagnostic GmbH manufactures instruction at 4000 rpm at 2- 8°C for 10 minute, the clear supernatant was separated by micropipette and then stored in a deep freeze at -20°C till further assessment for HGH level in saliva by Human Growth Hormone ELISA Kit; it's Catalog Number HGH0015BA Figure (1), by using Enzyme Linked–ImmunoSorbent Assay (ELISA) machine. However, the principle of reagent preparation, procedure assay and calculation of results were all done according to bioactiva diagnostica GmbH manufacture procedure instructions. In this method, the patient specimens (saliva) and control (containing the native HGH antigen) were first added to streptavidin coated wells. Biotinylated monoclonal and enzyme labelled antibodies are then added; these antibodies had high affinity and specificity and were directed against distinct and different epitopes of HGH. Reaction between the various HGH antibodies and native HGH occured in the microwells without competition forming a soluble sandwich complex. Simultaneously, the complex was immobilized in the well through the high affinity reaction of streptavidin and biotinylated antibody. The antibody-bound fraction was separated from unbound antigen by aspiration, then the enzyme activity horseradish peroxidase (HRP) present on the surface of the well was quantified by reaction with the TMB substrate to produce color. The enzyme activity in the antibody-bound fraction was directly proportional to the native antigen concentration.

Figure 1: Human Growth Hormone (HGH) **ELISA Kit**

For occlusal assessment, each child was examined with the teeth in centric occlusion; the terminal plane which is the relationship of the maxillary and mandibular second primary molars in the vertical plane was classified according to Baume [14], into three types:

A-Flush terminal plane: the distal surfaces of maxillary and mandibular primary second molars lie in the same vertical plane.

B- Distal-step: the distal surface of mandibular primary second molar is distal to that of the primary maxillary second molar.

C-Mesial-step: the distal surface of mandibular primary second molar is mesial to that of the maxillary primary second molar.

For Anthropometries measurements, the child age was calculated by subtraction his birth date from the date of the visit or measurement. To subtract, it was be necessary to convert months to days and years to months if either the month or day in the birth data was larger than in the date of measurements. When converting one month to days, subtract 1 from the number of months in the date of measurement, then add 28, 30, or 31, as appropriate, to the number of days. When converting one year to months, subtract 1 from the number of years in the date of measurement, then add 12 to the number of months, the age was determined age to the nearest 1/4 year [15], the child height recorded according to Trowbridge [16], the index for measuring children's growth problems used in this study was Height for Age Z-score (HAZ) which was calculated according to Centers for Disease Control and Prevention (CDC) [17], HAZ scores which also called standard deviation (SD) of < -2 were considered to fulfill the criterion for stunting whereas SD of < -3 were defined as sever stunting.

3. Results

Table (1) shows the difference in salivary HGH concentration (pg/ml) between preschool children with 7-9 sleeping hr./day and preschool children with 10-12 sleeping hr./day.

Results reported that the mean value of salivary HGH was lower among children with 7-9 sleeping hr./day than children with 10-12 sleeping hr./day with statistically highly significant difference (P< 0.01). Concerning each gender the same highly significant difference finding was recorded (P< 0.01).

Table (2) illustrates the relation of the salivary HGH concentration with sleeping hr./day of preschool children.

For preschool children with 7-9 sleeping hr./day, the relation between the salivary HGH concentration and sleeping hr./day was strong highly significant in positive direction. For preschool children with 10-12 sleeping hr./day, the relation between the salivary HGH concentration and sleeping hr./day was strong highly significant in positive direction . For total preschool children, the relation between the salivary HGH concentration and sleeping hr./day was also strong highly significant in positive direction.

Figure (2) illustrates the distribution of preschool children according to the terminal plane type in relation to sleeping hr./day. This figure shows that 35.8% of preschool children with 7-9 sleeping hr./day had a distal step, followed by 12.6% and 1.3% of preschool children had flash terminal plane and mesial step respectively, while 33.8% of preschool children with 10-12 sleeping hr./day had a mesial step, followed by 10.6% and 5.3% preschool children had flash terminal plane and distal step respectively.

Table (3) shows the difference in HAZ between preschool children with 7-9 sleeping hr./day and children with 10-12 sleeping hr./day.

The results reported that the mean value of HAZ was highly significantly lower among children with 7-9 sleeping hr./day than children with 10-12 sleeping hr./day(P< 0.01). Concerning each gender the same finding was recorded with statistically highly significant difference (P< 0.01).

Table (4) demonstrates the relation of the HAZ with sleeping hr./day for preschool children.

For preschool children with 7-9 sleeping hr./day, the relation between HAZ and sleeping hr./day was weak non-significant in positive direction.

For preschool children with 10-12 sleeping hr./day, the relation between HAZ and sleeping hr./day was weak non-significant in negative direction. For total preschool children, the relation between HAZ and sleeping hr./day was strong highly significant in positive direction.

Figure (3) illustrates the distribution of preschool children according to the HAZ percentage in relation to sleeping hr./day.

Concerning preschool children with 7-9 sleeping hr./day, the percentage of HAZ above average (above 1SD); with average (0SD), and below average (below -1SD) were 13.3%, 50.7% and 17.3 respectively, with 13.3% and 5.3% children were suffer from stunting and sever stunting (below -2SD and -3SD of the reference CDC population) respectively. Concerning preschool children with 10-12 sleeping hr./day, the percentage of HAZ above average (above 1SD); with average (0 SD), and below average (below-1SD) were 41.3%, 56% and 2.7% respectively, with no children were suffer from stunting or sever stunting.

Table 1: Difference in salivary HGH concentration (pg/ml) between preschool children according to sleeping hr./day

Variables	Genders	7-9 Sleeping hr./day		10-12 Sleeping hr./day		Difference	
		No.	Mean± S.D	No.	Mean± S.D	T-Test	p-value
Salivary HGH	Boys	20	.864±.13	20	5.33±.87	-22.46	.000**
	Girls	20	.878±.07	20	5.19±.91	-21.01	.000**
	Total	40	.871±.10	40	5.26±.88	-31.03	.000**

** HS: Highly Sig. at P<0.01 between two groups children.

Table 2: Correlation between salivary HGH concentration (pg/ml) and sleeping hr./day among preschool children

	7-9 Sleeping hr./day		10-12 Sleeping hr./day		Total sample	
	r	p-value	r	p-value	r	p-value
Salivary HGH (pg/ml)	.732	.000 **	.967	.000 **	.956	.000**

** HS: Highly Sig. at P<0.01 between two groups children, r= (Person's correlation coefficient).

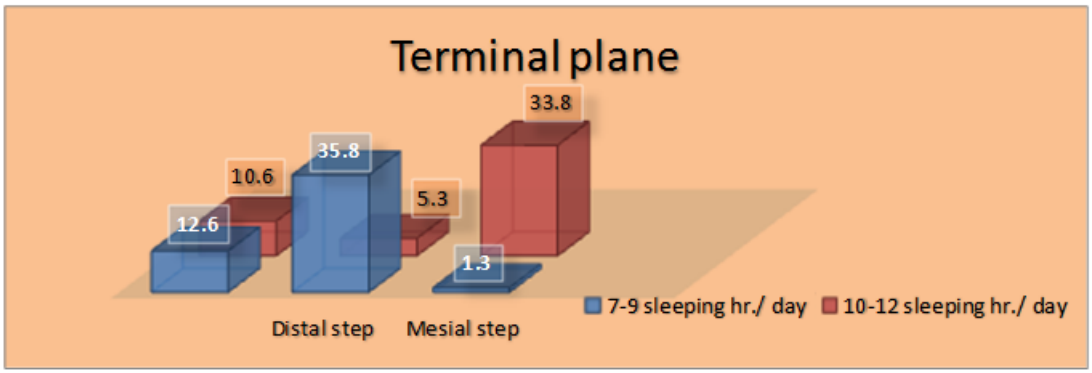

Figure 2: Distribution of preschool children according to the terminal plane type in relation to sleeping hr./day

Table 3: Difference in HAZ between preschool children according to sleeping hr./day

	7-9 Sleeping hr./day		10-12 Sleeping hr./day		Total sample	
	r	p-value	r	p-value	r	p-value
HAZ	.002	.984	-.124	.288	.391	.000**

** HS: Highly Sig. at P<0.01 between two groups children, (HAZ) Height for Age Z-score.

Table 4: Correlation between HAZ and sleeping hr./day among preschool children

Variables	Genders	7-9 sleeping hr./day		10-12 sleeping hr./day		Difference	
		No.	Mean± S.D	No.	Mean± S.D	T-Test	p-value
HAZ	Boys	38	-.50±1.15	37	.49±.50	-4.761	.000**
	Girls	37	-.43±.95	38	.29±.56.	-3.986	.001**
	Total	75	-.47±1.05	75	.39±.54	-6.220	.000**

Non Sig. at P>0.05; ** HS: Highly Sig. at P<0.01 between two groups children, r= (Person's correlation coefficient), (HAZ) Height for Age Z-score

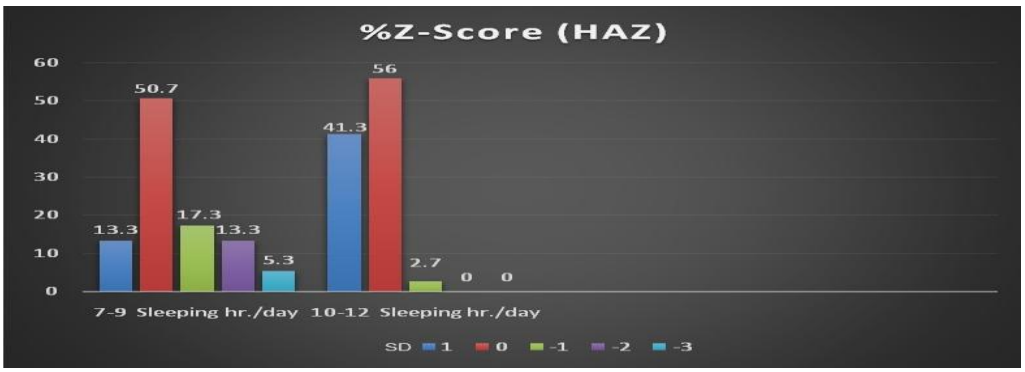

Figure 3: Distribution of preschool children according to the HAZ percentage in relation to sleeping hr./day

4. Discussion

In the present study, sandwich Enzyme-Linked Immuno Sorbent Assay (ELISA) was used for salivary HGH concentrations measurement as it is a diagnostic tool in medicine because of its advantages as compared to other immunoassay methods, which is more sensitive, accurate, specific, fast since many samples can be processed at once—about 90 samples per plate in 2-3 hours, very little sample volume is needed less than 100 µl in most cases, easy to learn and simple procedures [(18)].

No previous study measured HGH concentration in the saliva; the present study represents the first study, therefore it is difficult to compare the salivary HGH of the present study with those studies which measured it in the serum of children because of the wide variation in the studies design, inclusion criteria, number of patients, children age, in addition there was no agreement on the best testing method of HGH levels in children, different immunoassay kits use different methods to measure serum levels and, therefore, produce different results [(19)].

The mean value of salivary HGH of the current study was significantly lower among children with 7-9 sleeping hr./day than children with 10-12 sleeping hr./day that could be explained by the fact that the sleep deprivation and irregular sleep habits can alter hypothalamus and pituitary function, which results in changes in levels of several hormones including leptin, ghrelin, insulin, cortisol, and growth hormone [(20)], that the majority bulk of the HGH pulse secretion occurs shortly after sleep onset and the peak

of HGH secretion occur during sleep at night, so altering in sleep-wake cycle could lead to HGH release inhibition [21]. In addition, melatonin hormone which is secreted during sleep at night inhibits osteoclast activity and vice versa for osteoblast activity which induce bone growth [22]. These findings explained the strong highly significant correlation between sleep duration and HGH and children growth which represented by HAZ.

These findings revealed the importance of regular daily sleep pattern for both physical and mental development in children since the majority of parents, particularly parents of children with growth problems complaint about the time of their children going to sleep at night.

The further explanation to the current results of 13.3% and 5.3% children with 7-9 sleeping hr./day who were suffer from stunting and sever stunting, that there were many other factors might affect children's growth such as genetic; non genetic factors and environmental factors [23]; genetic factors such as parent's height which might have direct influence on children's growth and could predict their adulthood height , one limitation of the current study that parent's height didn't take into consideration in this study. Non genetic factors such as birth weight, diseases, and nutritional status also might affect children's growth [24, 25]. Nutritional status is the result of a complex interaction between the food, overall health, and the environment [23]. In Iraq, the environmental factors and unstable security situation particularly in Baghdad after 2003 war and following years were highly associated with child nutritional status, families who lived in non secure area have 2.3 times more opportunities to have malnourished child [26], or this result might be due to sample size, study design and children age

The indicator HAZ was used in this study as it is a measure of how many standard deviations the individual's height is from references CDC population, age specific data (2-20 years) came from the Centers for Disease Control and Prevention [17, 27].

In this study the selected age of children was 5 years as the entire primary teeth were completely erupted in the oral cavity in order to exclude the mixed dentition dynamic period, this study was conducted to reveal the effect of sleep on body growth and occlusion because if malocclusion was observed in the primary dentition, it was to be expected that the same irregularities will occur in the corresponding permanent dentition only to a more pronounced degree [28].

The results of the present study also showed that the most common terminal plane among children with 7-9 sleeping hr. /day was the distal terminal plans followed by flush terminal plane and mesial terminal plane respectively, this result could be explained by the fact that the craniofacial and dental growth as with somatic growth are dependent on a normal hormonal milieu so any deficiencies in HGH or insulin-like growth factor I (IGF-I) during childhood cause diminished growth of the facial bones, including the maxilla and extremely deficient growth of the mandible to a greater degree, resulting in a retrognathic relationship [29, 30].

Generally, it is difficult to compare the results of this study with other study taking the same subject because the variability in study design, sample size, age, selection criteria, geographical area, living environment, length of observation and diet.

The results of the present study showed that the most common terminal plane among children with 10-12sleeping hr./day was the mesial terminal plane followed by flush terminal plane and distal terminal plane respectively, this mesial terminal plane was found to be the normal phenomena completed primary dentition rather than flush terminal plane [28]. This result showed that the majority of the preschool children with 10-12 sleeping hr./day will have favorable permanent molar relation which considered and ideal concept for transition to class I Angle's molar relation in permanent dentition [31]. In conclusion, children with 10-12 sleeping hr./day had fewer deviation from normal occlusion which indicates decrease tendency for malocclusion in permanent dentition.

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