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ORIGINAL ARTICLE

A prospective longitudinal study to estimate the prevalence of obesity in Egyptian children with nocturnal enuresis and the association between body mass index and response to therapy

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KEYWORDS

Behavioral problems;
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Response to treatment

Abstract *Purpose:* Nocturnal enuresis is defined as the involuntary voiding of urine in bed beyond the age at which bladder control is normally obtained. Previous studies have suggested a high rate of obesity in children with nocturnal enuresis. We evaluated this hypothesis and investigated the correlation between body mass index and the efficacy of treatment.

Subjects and methods: This was a prospective–longitudinal study done on 180 children with primary mono symptomatic nocturnal enuresis. Urinary diary data and body mass index percentile were determined. Response to the treatment was evaluated statistically and correlated with body mass index percentile. Response to different modalities of the treatment were also statistically evaluated separately and correlated with the BMI. The statistical test used was ANOVA *F*-test.

Results: 22.7% of our patients with nocturnal enuresis were either overweight or obese (13.9% and 7.8% respectively) according to the Egyptian national growth charts. Therefore, the prevalence of overweight and obesity is not higher in children with nocturnal enuresis as compared to the overall prevalence in normal Egyptian children (14%). But when we adjusted these values according to the social class of our patients, the prevalence of obesity was higher than normal for children of low socioeconomic status.

There was no significant correlation between response to standard pediatric urological interventions (behavioral therapy, alarm therapy, imipramine, desmopressin, oxybutynin and tolterodine) and the BMI of our patients. Also, there was no significant difference in the response or relapse rates to the different modalities of therapies in the groups of patients with different BMI.

Conclusions: Obesity correlates with high BMI in lower socioeconomic classes, but does not correlate with efficacy of the treatment in children with nocturnal enuresis.

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1. Introduction

Primary mono symptomatic nocturnal enuresis (PMNE) is one of the most common developmental problems in children which affects up to 20% of children at five years of age and nearly 1% of young adults [1]. It is defined as involuntary urination while asleep after the age at which bladder control usually occurs [2]. Mono symptomatic enuresis is defined as enuresis in children without any other urinary tract symptoms and without a history of bladder dysfunction [2].

Obesity is increasing at an alarming rate throughout the world. Obesity is a condition of excess body fat often associated with a large number of debilitating and life-threatening disorders. Obese children are at a higher risk for asthma and obstructive sleep apnea during childhood, as well as hypertension, diabetes, cardiovascular disease, gall bladder disease and osteoarthritis in adulthood [3,4]. Overweight children have an increased risk of being overweight as adults [3].

A good understanding of the association between obesity and socioeconomic status (SES) has many important public health and policy implications. In the developed countries there is a strong inverse relationship between obesity and SES. While in the developing societies a strong direct relationship exists between SES and obesity among men, women and children [5].

Previous studies suggested a high rate of obesity in children with various forms of dysfunctional voiding, especially nocturnal enuresis [6]. However, there are few data regarding whether obese children with nocturnal enuresis are more or less likely to respond to the treatment. In a study done by Ahmet et al. [7] a better response to the standard treatment in patients with a BMI below the 85th percentile was seen. They speculated that the lower success rates for the treatment in patients with high BMI suggest not only that there is an association, but also that obesity and incontinence may well share a common etiology.

In the current study, we investigated the prevalence of obesity among Egyptian children with primary mono symptomatic nocturnal enuresis. And we investigated the correlation between BMI and efficacy of the treatment. We also correlated the BMI to the response and relapse rates of the different standard pediatric urological interventions used for the treatment of enuresis in an attempt to identify the best modality of therapy that can be used for the treatment of obese or overweight enuretic children in Egypt.

2. Patients and methods

This was a prospective–longitudinal study. We evaluated 400 children (age ranges from 5–16 years) chosen randomly from our Child and Adolescence Psychiatry Clinic, Children Hospital, Ain Shams University who had a diagnosis of primary mono symptomatic nocturnal enuresis (PMNE) according to the Standardization Committee of the International Children's Continence Society [2] in the period between July 2012 and July 2014.

One hundred and eighty patients met our criteria. Eligible patients had significant nocturnal enuresis (non diurnal) without any other urinary tract symptoms and without a history of bladder dysfunction with wetting frequency of 3 or more nights per week, for at least 2 weeks of observation.

Patients with one or more of the following were excluded from the study; diurnal enuresis, urinary tract infection, polyuric disorders (diabetes mellitus or diabetes insipidus), abnormal urine analysis, urinary tract abnormality, significant hydronephrosis or reflux, history of renal disease, hypertension or genitourinary abnormality, neurological disease, mental retardation or psychological disease. Those with secondary enuresis were, also, excluded (i.e. children who develop enuresis after a dry period of six months or more [2]).

3. Methodology

Each patient in this study was subjected to the following; Full *history* was taken from all patients, including; demographic data, presence of organic or psychological diseases, family history of similar cases, and previous treatment for enuresis which was received. Also assessment of the socioeconomic status of the patients was done.

Clinical examination including; body measurements, physical examination and neurological examination.

Weight (*Wt*)*:* The body weight was measured using Seca scale and recorded to the nearest 0.1 kg. The measures were converted into percentiles and were entered separately to the Egyptian growth charts for age and sex [8].

Height (*Ht*)*:* The standing height was measured using stadiometer to the nearest centimeter. The measures were converted into percentiles and were entered separately to the Egyptian growth charts for age and sex [8].

Body Mass Index (*BMI*): Body mass index was calculated as weight in kilograms divided by the square of height in meters (Wt (kg)/Ht (m^2)). Then, it was plotted by age on a gender specific Egyptian growth chart [8] to determine the child's category, which may be one of the following four categories [9]*:*

- Normal weight: between the 5th and the 85th percentiles.
- Overweight: between the 85th and 95th percentiles.
- Obese: higher than 95th percentile.
- Underweight: less than 5th percentile.

Investigations: Urine analysis (for all cases): To exclude presence of pyuria, glucosuria and diabetes insipidus.

Scales: The *Stanford-Binet Intelligence scale* [10] was used for all the participating children to determine intelligence quotient or IQ which is simply the ratio of mental age (MA) to chronological age (CA) multiplied by 100 (IQ = MA/CA × 100). The Stanford–Binet Intelligence Scale consists of 15 subsets. The subsets are grouped into 4 broad areas verbal reasoning, abstract/visual reasoning, quantitative reasoning and short term memory and a composite score is derived from the area scores.

The Socioeconomic Status Scale for health research in Egypt-the new version [11] was performed for all patients and their families. This scale was developed and validated by Fahmy and El-Sherbini [12] and updated and re-validated in 2012. The new socioeconomic status scale has 7 domains (education and culture, occupation, family, family possessions, economic, home sanitation and health care) with a total score of 84, with a higher score indicating better SES. Socioeconomic level is classified into very low, low, middle and high levels depending on the quartiles of the score calculated.

After the initial evaluation of the bladder behavioral therapy was given to all children. These behavioral instructions included increasing early daytime fluids, timed frequent voiding, reducing fluid intake in the evening and voiding before bed [13]. Voiding diaries were used for the evaluation of treatment progress (number of accidents, nocturia, presence of urinary symptoms or constipation if any).

A written consent was obtained from the parents of all the children participating in the study and approval from the Ain Shams University Ethics committee was obtained. Also, the work has been carried out in accordance with the code of Ethics of the World Medical Association (Declaration of Helsinki) for the experiments involving humans.

The choice of treatment modality prescribed for each patient was chosen randomly using a basic numbered ball technique. Depending on the treatment modalities patients were using, children were divided into six groups. The number of patients included in each group was pre decided (depending on the medication availability in our clinic).

Group 1: (70 patients-behavioral therapy group) children were on behavioral therapy as the only modality of therapy.

Group 2: (20 patients-desmopressin group) children in this group were on DDAVP tablets (desmopressin tablets 0.2 mg *Minirin*™, Ferring International Centre, Switzerland) once daily before bedtime in addition to behavioral instructions.

Group 3: (20 patients-tofranil group) they were on combined imipramine, the antidepressant (Tofranil™ tablets 25 mg) taken orally one hour before bed-time and behavioral therapy.

Group 4: (20 patients-uripan group) they were on combined oxybutynin-nonselective anticholinergic (Uripan™ syrup 5 mg) taken orally one hour before bed-time and behavioral therapy.

Group 5: (20 patients-tolterodine group) they were on combined Tolterodine™-selective anticholinergic (tablets 2 mg) taken orally one hour before bed-time and behavioral therapy.

Group 6: (30 patients-alarm group) These patients used Wet Stop PALCO™ (wet-stop Alarm; Palco Laboratories, USA). This is an enuretic alarm, details on the use of the alarm were given to the parent in addition to behavioral instruction.

Follow up visits for all groups were scheduled every 2 weeks for 6 months: To ensure adherence to behavioral instructions and drug or alarm therapy, to record any adverse effect, and to analyze the progress of the patients.

The main variable for analysis was the average number of wet nights per week calculated over a defined period of 24 weeks.

Treatment outcomes were defined as follows [14]: "*Complete responders*" were patients with 1 or less wet night per week in the last 2 weeks of the treatment. "*Partial responders*" were those with more than 1 wet night per week but greater than 50% reduction in the number of wet nights compared with the pre treatment period. "*Non-responders*" had less than 50% reduction in wetting frequency in the last 2 weeks of the treatment. Non-compliant patients and non-responders were considered together as "treatment failure".

Patients were followed for 12 weeks after cessation of the treatment. "*Relapsers*" were defined as reappearance of >1 wet night per week for complete responders or $>50\%$ of the pretreatment wetting frequency for partial responders. "*Early relapsers*" and "*late relapsers*" were defined as those who relapsed in the first 4 weeks and next 4 weeks of the post-treatment observation period, respectively.

3.1. Statistical methodology

The collected data were revised, coded, tabulated and introduced to Statistical package for Social Science (SPSS 15.0.1 for windows; SPSS Inc, Chicago, IL, 2001). Data were presented and suitable analysis was done according to the type of data obtained for each parameter.

For the descriptive statistical analysis of the subjects' demographic data (mean, standard deviation ($\pm$SD) and range for parametric numerical data, while median was used for non parametric numerical data and frequency and percentage of non-numerical data) we used the *Student's t-test* to assess the statistical significance of the difference between two study group means. *Mann–Whitney Test* (*U test*) was used to assess the statistical significance of the difference of a non parametric variable between two study groups.

For the analytical statistical analysis the *ANOVA test* was used to assess the statistical significance of the difference between more than two study group means.

4. Results

We evaluated 180 children (89 girls [49.4%] and 91Boys [50.6%]) with PMNE. Mean patient age was 9.12 $\pm$ 2.56 years ranging between 5 and 16 years. Nine patients (5%) were underweight, 132 patients (73.3%) were of normal weight, 25 patients (13.9%) were overweight and 14 patients (7.8%) were obese. All the patients had significant enuresis prior to the treatment. The mean number of wet nights per week was 6.1, 6.3, 6.7 and 5.8 in the underweight, normal weight, overweight and obese patients respectively.

There were no statistically significant differences based on age, gender, family history, number of wet nights per week, social class, or the treatment modalities between the BMI groups Table 1.

There was no statistically significant difference in the response to treatment or relapse rates in children with different BMI. However, there were minor differences (not reaching statistically significant values); for example a lower incidence of complete response was noted in the overweight patients. On the other hand, relapse rates (early and late) were more common in the underweight patients Table 2.

This study also showed that there was no statistically significant difference between the different groups of patients (divided according to the modality of therapy they were receiving) and the patients' BMI. However, there were some differences in the response pattern of each group.

In group 1 (behavioral therapy only); overweight patients were (18.6%) of the total number of patients, this is slightly more than the general percentages. They also show lower incidence of complete responders (7.7%) and higher incidence of non-responders (30.8%). In this group also obese patient showed a high incidence of relapse (40%). It is worth mentioning that this group compromised the largest percentage of patients (38.9%) Table 3.

In group 2 (desmopressin); there was no significant difference as regards response although the overweight patients showed no complete response but were divided between the partial and no response categories. The relapse was generally higher in this group than other groups reaching 75% between early and late relapse Table 4.

Table 1 Statistical comparison of patient characteristics with respect to the BMI.

Variable		BMI groups				X^2	P
		Underweight (N = 9)	Normal (N = 132)	Overweight (N = 25)	Obese (N = 14)		
Age in years		8.6 ± 2.7 (5–12)	9.4 ± 2.4 (5–16)	8.8 ± 3.2 (5–14)	8.2 ± 2.5 (6–15)	1.522**	.211
Sex	Male	4	69	10	6	1.648	.649
		44.4%	52.3%	40.0%	42.9%		
	Female	5	63	15	8		
		55.6%	47.7%	60.0%	57.1%		
Family history	Positive	4	57	6	7	2.913	.405
		66.7%	73.1%	66.7%	100.0%		
	Negative	2	21	3	0		
		33.3%	26.9%	33.3%	0.0%		
Number of wet nights per week prior to therapy		6.1 ± 1.5 (3–7)	6.3 ± 1.4 (3–7)	6.7 ± .8 (3–7)	5.8 ± 1.9 (3–7)	1.46**	.227
SES scale		14–28	16–30	18–29	20–30		
Treatment type	Behavioral therapy	4	48	13	5	13.917	.532
		44.4%	36.4%	52.0%	35.7%		
	Desmopressin therapy	2	15	2	1		
		22.2%	11.4%	8.0%	7.1%		
	Tofranil therapy	1	17	1	1		
		11.1%	12.9%	4.0%	7.1%		
	Uripan therapy	0	17	3	0		
		0.0%	12.9%	12.0%	0.0%		
	Tolterodine therapy	2	14	1	3		
		22.2%	10.6%	4.0%	21.4%		
	On alarm	0	21	5	4		
		0.0%	15.9%	20.0%	28.6%		

ANOVA (F test).

Table 2 Results of treatment in children with nocturnal enuresis with respect to the BMI.

Variable		BMI groups				X^2	P
		Underweight (N = 9)	Normal (N = 132)	Overweight (N = 25)	Obese (N = 14)		
Response	Non-responder	0	23	5	1	10.683	.099
		0.0%	17.4%	20.0%	7.1%		
	Partial response	4	38	12	2		
		44.4%	28.8%	48.0%	14.3%		
	Complete response	5	71	8	11		
		55.6%	53.8%	32.0%	78.6%		
Relapse	No relapse	6	92	18	11	1.237	.975
		66.7%	77.3%	81.8%	78.6%		
	Early relapse	2	16	3	2		
		22.2%	13.4%	13.6%	14.3%		
	Late relapse	1	11	1	1		
		11.1%	9.2%	4.5%	7.1%		

ANOVA (F test).

Group 3 (tofranil); most patients in this group were complete responders (70%) with low incidence of relapse (15% early and 5% late relapse) Table 5.

Group 4 (uripan); in this group there were neither underweight nor obese patients. Moreover, most overweight patients showed partial response to this particular modality of therapy (66% of overweight patients) associated with high incidence of late relapse (33% of overweight patients) Table 6.

Group 5 (tolterodine); obese patients (15%) in this group were more than the general percentages Table 7.

Group 6 (alarm); this group shows very high incidence of complete responders in all BMI groups (87% of the patients). There was also no relapse seen in this particular group Table 8.

5. Discussion

According to our study results, the prevalence of overweight and obesity (13.9% and 7.8%, respectively) is not higher in children with nocturnal enuresis than their prevalence in the normal Egyptian population. The prevalence of overweight

Table 3 Results of treatment in children of group 1 (receiving behavioral therapy only) with respect to the BMI.

Variable		BMI groups				X^2	P
		Underweight (n = 4)	Normal (n = 48)	Overweight (n = 13)	Obese (n = 5)		
Response	Non-responder	0	12	4	0	7.568	.271
		0.0%	25.0%	30.8%	0.0%		
	Partial response	2	23	8	2		
		50.0%	47.9%	61.5%	40.0%		
	Complete response	2	13	1	3		
		50.0%	27.1%	7.7%	60.0%		
Relapse	No relapse	4	31	9	3	4.207	.649
		100.0%	77.5%	81.8%	60.0%		
	Early relapse	0	7	2	1		
		0.0%	17.5%	18.2%	20.0%		
	Late relapse	0	2	0	1		
		0.0%	5.0%	0.0%	20.0%		

ANOVA (F test).

Table 4 Results of treatment in children of group 2 (receiving desmopressin and behavioral therapy) with respect to the BMI.

Variable		BMI groups				X^2	P
		Underweight (N = 2)	Normal (N = 15)	Overweight (N = 2)	Obese (N = 1)		
Response	Non-responder	0	5	1	0	4.215	.648
		0.0%	33.3%	50.0%	0.0%		
	Partial response	1	3	1	0		
		50.0%	20.0%	50.0%	0.0%		
	Complete response	1	7	0	1		
		50.0%	46.7%	0.0%	100.0%		
Relapse	No relapse	0	5	0	0	6.020	.421
		0.0%	50.0%	0.0%	0.0%		
	Early relapse	1	2	1	1		
		50.0%	20.0%	100.0%	100.0%		
	Late relapse	1	3	0	0		
		50.0%	30.0%	0.0%	0.0%		

ANOVA (F test).

Table 5 Results of treatment in children of group 3 (receiving imipramine and behavioral therapy) with respect to the BMI.

Variable		BMI groups				X^2	P
		Underweight (N = 1)	Normal (N = 17)	Overweight (N = 1)	Obese (N = 1)		
Comment at 8 weeks	Non-responder	0	1	0	0	1.513	.959
		0.0%	5.9%	0.0%	0.0%		
	Partial response	0	5	0	0		
		0.0%	29.4%	0.0%	0.0%		
	Complete response	1	11	1	1		
		100.0%	64.7%	100.0%	100.0%		
Relapse	No relapse	0	14	1	1	6.324	.388
		0.0%	82.4%	100.0%	100.0%		
	Early relapse	1	2	0	0		
		100.0%	11.8%	0.0%	0.0%		
	Late relapse	0	1	0	0		
		0.0%	5.9%	0.0%	0.0%		

ANOVA (F test).

Table 6 Results of treatment in children of group 4 (receiving oxybutynin and behavioral therapy) with respect to the BMI.

Variable		BMI		X^2	P
		Normal (n = 17)	Overweight (n = 3)		
Comment at 8 weeks	Non-responder	1 5.9%	0 0.0%	3.305	.192
	Partial response	3 17.6%	2 66.7%		
	Complete response	13 76.5%	1 33.3%		
Relapse	No relapse	12 70.6%	2 66.7%	.672	.715
	Early relapse	2 11.8%	0 0.0%		
	Late relapse	3 17.6%	1 33.3%		

ANOVA (F test).

Table 7 Results of treatment in children of group 5 (receiving tolterodine and behavioral therapy) with respect to the BMI.

Variable		BMI		X^2	P
		Normal (n = 17)	Overweight (n = 3)		
Comment at 8 weeks	Non-responder	1 5.9%	0 0.0%	3.305	.192
	Partial response	3 17.6%	2 66.7%		
	Complete response	13 76.5%	1 33.3%		
Relapse	No relapse	12 70.6%	2 66.7%	.672	.715
	Early relapse	2 11.8%	0 0.0%		
	Late relapse	3 17.6%	1 33.3%		

ANOVA (F test).

in Egypt according to the WHO is 14% of normal children [15].

An Egyptian study made by Badawi et al. [16] showed that the prevalence of overweight and obesity was 17.7% and 13.5% respectively in normal Egyptian children of different socioeconomic status.

The result of almost similar prevalence of overweight and obesity among both enuretic and normal children comes in concordance with another study made by Burgu et al. [17]. But, contrary to our results Bakry et al. [18] reported a positive statistically significant relationship between over-weight and nocturnal enuresis in Egyptian children aged 4–12 years.

However, we must draw attention that although our results show a similarity in the prevalence of overweight and obesity between children with nocturnal enuresis and normal children in the overall general Egyptian population, the prevalence of overweight and obesity in the studied enuretic children is actually higher than normal for children of low socioeconomic status attending public schools from whom our study population was derived. Measure of the socioeconomic status done for our patients (made in the beginning of the study) showed that all included patients fell in the very low and low socioeconomic class category. Included patients were gathered from the attendee of the Child and Adolescent Psychiatry Clinic, Children hospital, Ain Shams University (where the study was performed) which is a governmental nonprofit institute providing free service for mostly under privileged patients attending governmental schools. This explains why our patients, exclusively, were in the lower socioeconomic class.

Confirming the hypothesis that obesity is less common among children of low socioeconomic class, is a study mediated by Hafez et al. [19] which showed a prevalence of overweight and obesity of 11% and 3.8% respectively, among healthy underprivileged children of governmental school in Cairo. On the other hand higher values were detected among children of private schools with percentage values of 21% and 12.9% for overweight and obesity, respectively.

Moreover, a review of 144 published studies about the relationship between socioeconomic status (SES) and obesity reveals a strong direct relationship between SES and obesity among men, women, and children in the developing countries. They suggested that parents with better economic means in the developing countries seem to indulge themselves and their children with more food [20].

Therefore if we made consideration for the socioeconomic status of our patients, then we may suggest that the prevalence of overweight and obesity is higher in our enuretic patients than the normal Egyptian population in this particular socioeconomic class.

The finding of higher prevalence of obesity among children with enuresis is supported by a study done by Erdem et al. [6] which noted that elimination disorders especially nocturnal enuresis were correlated with higher BMI.

Obese children may have hormonal differences, dietary differences and even personality/behavioral differences compared

Table 8 Results of treatment in children of group 6 (on alarm and behavioral therapy) with respect to the BMI.

			BMI groups			X^2	P
			Normal (N = 21)	Overweight (N = 5)	Obese (N = 4)		
Comment at 8 weeks		Non-responder	3 14.3%	0 0.0%	1 25.0%	1.257	.533
		Complete response	18 85.7%	5 100.0%	3 75.0%		
Relapse	No relapse	21 100.0%	5 100.0%	4 100.0%	–		–

ANOVA (F test).

to non obese children. Any of these factors could account for the increased rate of incontinence.

Sleep disordered breathing, a condition increasingly recognized in obese children [21] may explain some of the association between nocturnal enuresis and obesity. It has been observed that children with sleep disordered breathing have an increased incidence of nocturnal enuresis [22].

Moreover, there is the work done by Esposito et al. [23] to evaluate the presence of sleep disturbances in 190 children with affected PNE and 766 typically developing children. In this study they evaluated sleep habits and disturbances by Sleep Disturbances Scale for Children (SDSC) questionnaire filled by the mothers. The questionnaire consisting of six subscales: Disorders in Initiating and Maintaining Sleep (DIMS), Sleep Breathing Disorders (SBD), Disorders of Arousal (DA), Sleep–Wake Transition Disorders (SWTD), Disorders of Excessive Somnolence (DOES), and Nocturnal Hyperhidrosis (SHY). The result of the study showed that PNE children show a higher prevalence of all sleep disturbances. Therefore it was concluded that, PNE tends to alter sleep architecture and could be a risk factor or a consequence for the development of sleep disorders.

What is equally likely in our opinion is that parenting style in obese children might be counterproductive for treating incontinence. Rhee et al. [24] reported that a permissive, neglectful or restrictive parenting style increases the risk of obesity. These parenting styles could certainly be a causative factor for voiding problems as well.

In our study we did not find a statistically significant correlation between the responses to the different modalities of the treatment in patients with different BMI. Although a slight lower incidence of complete response was noted in the overweight patients, we searched the literature for studies that correlate the BMI of patients with nocturnal enuresis with the response to treatment. Few studies were found that address this particular issue. For example a study done by Ahmet et al. [7] found a better response to the standard treatment in patients with a BMI below the 85th percentile. They speculated that the lower success rates for the treatment in patients with high BMI suggest not only that there is an association, but also that obesity and incontinence may well share a common etiology.

For example obesity has been shown to be related to hormonal abnormalities in some children [4], and nocturnal enuresis may be related to an abnormality of antidiuretic hormone secretion [25]. Hence, a pituitary or other central nervous system abnormality could be the cause of both conditions.

Alternatively, a diet high in carbohydrates and fats and low in fiber, along with a sedentary lifestyle is likely to lead to obesity, as well as constipation and possible postponement of voiding [26].

When we correlated the BMI of our patients with the response and the relapse rates to the different modalities of the treatment used we found that there was no statistically significant difference between them. Therefore, any of the standard regimens used for the treatment of nocturnal enuresis can be used regardless the BMI of the patient.

6. Conclusions

In this study an association between obesity and an increased incidence of nocturnal enuresis was not confirmed in Egyptian children, but it is strongly suggested in patients with low socioeconomic status. In addition, we found no obvious correlation between BMI and the efficacy of treatment, nor did we find a correlation between BMI and the different modalities of therapy.

Author contribution

S.Z. worked on designing the work, acquisition of data, analysis and interpretation of data, drafting the article and revising it.

Conflict of interest

The author declares that there is no conflict of interest regarding the publication of this manuscript.

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