Middle East Journal of Applied Sciences Volume: 12 | Issue: 04| Oct. – Dec. | 2022

EISSN: 2706 -7947 ISSN: 2077- 4613 DOI: 10.36632/mejas/2022.12.4.38 Journal homepage: www.curresweb.com Pages: 544-552



Nutritional Assessment of Down Syndrome Children and Nutritional Awareness of their Caregivers in Delta Region, Egypt

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ABSTRACT

Background: Down's syndrome (DS) is the most common genetic condition in the world today and growth retardation is commonly seen in children with DS. Early identification of a growth problem is important because timely interventions may positively affect the child's general health and functional abilities as well as supporting growth. Children with DS have nutritional problems with unknown implications besides increased potential for obesity. The aim of study was to delineate eating and lifestyle habits of DS and assess dietary problems and challenges they face and to recommend appropriate monitoring strives to reduce the risk of inadequate nutrition, educate caregivers, and produce early detection and referral for conditions manifested by growth disorders. Methods: A cross sectional study was conducted including (106) child with DS including (64) males and (42) females attending the Intellectual Education Schools and follow up at outpatient clinic of Genetics department, Tanta Faculty of Medicine, Tanta University. An interview questionnaire was used including sociodemographic characteristics, dietary habits, and anthropometric measurements. Results: About (17.0%) of studies children were stunted, (13.2%) obese and (2.8%) were under weight. A significant positive correlation was found between the socioeconomic status of participants and awareness score of caregivers (r= 0.428, p= 0.000). Regarding energy intake and dietary macronutrients, energy intake exceeded the Estimated Energy proteins, fats, and carbohydrates Requirement corresponding to age, sex, body Excessive intake of vitamins B1, B2, and B6 was observed in all subjects, and that of vitamins B3 (niacin), B12, A, and C in the vast majority of subjects, while deficiency of vitamin D was observed in all individuals and folic acid in (22.2%) of individuals. Excessive intake of minerals, such as sodium, phosphorus, and was noted among most of the respondents, while calcium and magnesium were about half when compared to RDI% found to be insufficient. Conclusion: Our study can be valuable in the future interventions for the health care professionals, parents and caregivers of children and adolescents with DS in emphasizing the need for regular monitoring of their physical status and feeding behaviors.

Keywords: down syndrome, nutritional assessment, DRI, nutritional awareness

1. Introduction

Down Syndrome (DS) is a common gene alteration syndrome, mostly consisting of an anomaly in the 21^{st} chromosome. In Europe, DS children are 8% of registered cases of congenic anomalies. Worldwide, prevalence is 23 out of 10,000 births, with a tendency to increase (Roccatello *et al.*, 2021). Moreover, the chewing and swallowing difficulties, as well as delayed eruption of deciduous teeth, in children with DS which delay the introduction of solid foods additionally may cause nutritional deficiencies. Children with DS prefer simple carbohydrates and foods that are easy to chew and swallow, which results in the rejection of fresh fruits and vegetables from their diet (Skrzypek *et al.*, 2021).

The causes of abundant body mass in addition to genetic predisposition are inappropriate lifestyle behaviors, consuming too much energy for their needs as well as leading a sedentary lifestyle (Wernio *et al.*,2022).

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Inadequate nutrition is a disability-related problem for many children in developing countries, manifested as a lack of growth or stunting. The scope of this problem is substantial, with at least 30 per cent of children under the age of five reported being moderately or severely stunted (Wahdan and El-Nimr, 2018).

2. Methods

Across-sectional study was carried out among (106) patients (64 males and 42 females) diagnosed with DS. We used "Steven. Thompson" equation to calculate sample size (**Steven, 2012**). A personal interview was conducted asking children's parents or social specialist in Intellectual Education Schools in both Gharbia and El Behera governorates. Before participating in the study, the protocol was fully explained, and their informed consent was obtained.

Demographic and socioeconomic data assessment of participants were assessed by valid and reliable socioeconomic status scale for health research in Egypt, with permission of the original author. This scale has seven domains with a total score of (84) (El-Gilany *et al.*, 2012).

Medical Assessment was done including a detailed history, symptoms, extent of symptoms aggravation, genetics examination, chromosome karyotyping test, metabolic disorder hemoglobin analysis, and thyroid analysis for all cases.

Dietary intake was assessed by the 24-hour recall questionnaire which is one of the most reliable and widely used tools in nutritional assessment (Castell *et al.*, 2015). According to Food and Agriculture Organization criteria, the 24-hour dietary recall consists of recording all foods the patients eat, drinks, appetite, use of vitamins & minerals, number of meals and snacks, drinking coffee or any caffeine beverages and exercise for a 24-hour period for 3 consecutive days included weekend day to account the food fluctuations that occur during the week. Each subject was asked to inform us about all foods and drinks consumed in quantities and cooking method. Pictures of different sizes of food, standard cups and spoons have been used to help each respondent to estimate how much food the child ate (FAO, 2018). Data of the 24-hour food intake were coded; average of the three days was taken and entered to a computer program of food analysis. The analysis by this program based on food composition tables of Egyptian National Nutrition Research Institute (National Nutrition Institute2006). Then, percentage (%) of the mean daily intake of nutrients was calculated from the next formula: Factors such as age and gender were considered when assigning Dietary Reference Intakes (DRI) values for each respondent (Mahan and Escott-Stump, 2004).

Dietary assessment was also done using a valid and reliable scale and questionnaire which showed a respectable reliability in assessing the pupils' eating habits and identifying their attitudes towards food. The scale consists of 11 questions about important oral health-related eating habits. The score assigned to each response ranges from 1 to 4 with the maximum score assigned to the healthiest one. Hence, the total score is 44 (Turconi *et al.*, 2008).

Dietary awareness of caregivers: was assessed using the Nutrition Knowledge questionnaire. It contains eleven questions, each with four response categories structured in different ways. This section focused on a few nutritional aspects, investigating the level of nutritional awareness of their caregivers. The response categories are four and the true response of each question received a score of (1 and 0) for the other response. The total score of this section was (11) (Turconi *et al.*, 2003).

Data Processing and Analysis: Data were analyzed using SPSS version 25 (SPSS Inc., Chicago, IL, USA 1994). Quantitative data were presented as Mean \pm SD, median and interquartile range and qualitative data were presented as frequencies and percentages. The chi-square and Monte Carlo exact tests were used to assess associations between qualitative variables while the Mann-Whitney U test and the Kruskal-Wallis H test were carried out to compare quantitative variables among the different groups. Spearman correlation was used to correlate the awareness score of caregivers of DS children, dietary habits score and socioeconomic status among studied groups. The level of significance was considered at p value (<0.05).

3. Results

Table (1) show there is a non-statistically significant difference between anthropometric measures of studied Down syndrome children according to gender. Height for age in children with DS showed that more children (79.2%) were normal, and (17.0%) were stunted, while fewer children

(3.8%) were tall. Also, Obesity was recognized in (13.2%) of subjects and (2, 8%) were under weight and most children (84.0%) had normal weigh Head circumference for age was presented in table (1). It was found that, there is a non-statistically significant differences between male and female, (76.5% and 78.6% respectively) were had normal head circumference. While (6.3% and 2, 4% respectively) male and female had small head circumference. Also, (17.2% and 19% respectively) male and female had large head circumference.

gender (N=106)								
Variables	Total (n=106)		Male (n=64)		Female (n=42)		Test of	Р
	Ν	%	Ν	%	Ν	%	significance	value
Height for age:								
Normal	84	79.2	47	73.4	37	88.1	χ ² =4.832	0.075
Stunted	18	17.0	15	23.4	3	7.1		
Tall	4	3.8	2	3.1	2	4.8		
Range	85-166		85-10	56	90-15	0	-	
Mean ±SD	127.6±	19.7	130.2	2±21.2	123.7	±16.7		
Weight for age:								
Normal	89	84.0	53	82.8	36	85.7	$\chi^2 = 1.657$	0.543
Underweight	3	2.8	1	1.6	2	4.8		
Obese	14	13.2	10	15.6	4	9.5		
Range	12-140		12-14	40	13-13	8	-	
Mean ±SD	43.3±2	4.9	45.4=	⊧24.3	40±25	5.8		
Median (IQR)	35(25-	55.3)	41(20	6-60)	30(23	.8-50.8)		
Head circumference for age	:							
Normal	82	77.4	49	76.5	33	78.6	$\chi^2 = 0.866$	0.751
Small	5	4.7	4	6.3	1	2.4		
Large	19	17.9	11	17.2	8	19.0		
Range	44-70		44-70)	45-55		-	
Mean ±SD	50.8±3	.4	51.2=	⊨ 3.8	50.2±	2.7		
Median (IQR)	50(49-3	53)	50.5((49-53)	50(48	-52)		
Waist circumference:								
Range	51-140		53-12	26	51-14	0	Z=-1.418	0.156
Mean ±SD	77.3±1	8.4	78.8=	±17.2	75.2±	20.1		
Median (IQR)	75(63-	89.3)	76(60	5-91)	68(61	.8-82.3)		
Hip circumference:								
Range	50-138		50-12	28	54-13	8	Z=-1.486	0.137
Mean ±SD	81.3±1	8.2	82.4=	⊧17.1	$79.5\pm$	19.9		
Median (IQR)	79(67.8	8-93.3)	81.5((70-94.8)	74.5(6	55.8-92)		
BMI:								
Range	11.75-6	54	15-64	4	11.8-4	46	Z=1.769	0.076
Mean ±SD	24.9±9	.6	26.3=	10.3 ⊧	22.9±	8.1		
Median (IOR)	22.8(17	7.7-29.9)	23(19	9.5-32)	21.2(1	16.4-27.1)		

 Table 1: Distribution of anthropometric measures of studied Down syndrome children according to gender (N=106)

Table 2 show statistically significant correlation (r=0.457, p 0.008) regarding relation between dietary habits score of DS children and socioeconomic status statistically

 Table 2: Correlation between the awareness score of caregivers of DS children, dietary habits score and socioeconomic status

Maniahlan	Awaren	less score	Dietary habits score	
variables	r	p value	r	p value
Socioeconomic status	0.428	0.000*	0.257	0.008*
Dietary habits score	0.159	0.104		-

Figure (1) shows statistically significant correlation (r=0.428, p 0.000) regarding the correlation between the awareness score of caregivers of DS children and socioeconomic status



Fig. 1: Correlation between the awareness score of caregivers of DS children and socioeconomic status

Table (3): show that percentage of the DRI is taken, and more from (Caloric, Protein, Fat, and Carbohydrate). The proportions were as follows (174.6±65.1, 135.1±47.1, 208.5±100.6, 177.1±.68 9)

Table 3: Actual intake, DRI and % I	RI of calories and macronutrients among studied Down syndrome
children (N=106)	

	0)			
Variables	Intake	DRI	% DRI	
Caloric intake(kcal)				
Range	791.3-4853.7	620-2743	63-458	
Mean ±SD	20802.9±805.5	1248.3±.392 4	174.6±65.1	
Median (IQR)	1961.9(1554.2-2341.9	1097.5(958.3-1526)	163.4(130.9-212.5)	
Protein(gm)				
Range	27.3-205.2	31-134	19-288	
Mean ±SD	82.8±31.5	70.4 ± 37.3	135.1±47.1	
Median (IQR)	80.9(62.6-93.9)	54.6(47.876 8)	130.6(100.2-162.8)	
Fat(gm)				
Range	21.5-246.2	17-103	84-837.3	
Mean ±SD	68.9±33.8	35.1±12.9	208.5±100.6	
Median (IQR)	64.6(47.9-81.3)	30.3(26.2-42.6)	187.7(144.9-251.8)	
Carbohydrate(gm)				
Range	102.3-815.9	85-377	44.1418 9	
Mean ±SD	285.1±115.2	170.6±.53 7	177.1±.68 9	
Median (IQR)	256.1(200-341.1)	150.1(130.1-208.5)	167.9(121.9-220.6)	
SD= Standard Deviation IOR=Interguartile Range				

Deviation IQR=Interquartile Range

Table (4): shows a remarkable increase in the actual intake of sodium, potassium, iron, zinc and copper, while remarkable decrease in the actual intake of calcium and magnesium compared to the DRI.

Where the actual intake of sodium is 7 times, while the actual intake of calcium and magnesium is about half when compared to RDI%

Variables	Intake	DRI	% DRI
Sodium(mg)			
Range	865.9-6845.4	400-500	216.5-1487.8
Mean ±SD	3290.4±1158.9	455.7±49.9	725.4±249.2
Median (IQR)	3114.6(2512.2-3826.7)	500(400-500)	709.5(530.0-850.7)
Potassium(mg)			
Range	963.3-6291.7	1600-2000	60.2-341.0
Mean ±SD	2693.4±968.9	1822.6±199.6	147.7±50.6
Median (IQR)	2593.1(2054.5-3250.5)	2000(1600-2000)	142.1(118.3-169.4)
Calcium(mg)			
Range	207.9-1709.7	800-1300	15.9-205.6
Mean ±SD	605.4±266.3	1144.3±232.6	55.2±27.0
Median (IQR)	541.4(422.4-725.5)	1300(800-1300)	47.9(36.4-68.3)
Phosphorus(mg)			
Range	408.6-2892.7	500-1250	45.5-528.7
Mean ±SD	1214.6±457.7	733.5±348.9	193.3±93.3
Median (IQR)	1188.2(922.8-1348.8)	500(500-1250)	189.6(120.6-244.9)
Magnesium(mg)			
Range	43.8-339.7	130-410	17-222.26
Mean ±SD	111.1 ± 48.9	258.7±111.9	50.7 ± 30.8
Median (IQR)	103.5(79.1-130.9)	240(130-410)	43.6 (31-63)
Iron(mg)			
Range	5.4-45.4	8-11	56.6-413.8
Mean ±SD	16.3±6.6	$8.9{\pm}1.4$	184.2 ± 70.9
Median (IQR)	15.4(12.2-19.4)	8(8-11)	168.3(143.7-211.3)
Zinc(mg)			
Range	4.1-30.3	8-11	51-321
Mean ±SD	11.9±4.9	8.9±1.4	135.6±54.3
Median (IQR)	11.1(9.3-13.4)	8(8-11)	128(102-157.3)
Copper(mcg)			
Range	270-4130	440-890	61.3-677.2
Mean ±SD	1304.8±635	658.5±154.6	206.4±111.5
Median (IQR)	1195 (887.5-1545)	700(440-700)	180 (135-235.5)

 Table 4: Actual intake, DRI and % DRI of micronutrients among studied Down syndrome children (N=106)

SD= Standard Deviation IQR=Interquartile Range

Table (5): show that Actual intake more than, DRI and % DRI was taken in excess from Water (167.5 \pm 45.6) while Actual intake less than, DRI and % DRI from Fibers(32.8 \pm 14.4).

(N=106)			
Variables	Intake	DRI	% DRI
Water(ml)			
Range	1355.8-4193.1	1100-3900	63-307
Mean ±SD	3080.6±543.9	1930.6±.478 1	167.5±45.6
Median (IQR)	3159.3(2758.1-3485.5	1790(1600-2185)	170.8(137.7-200.9)
Fibers(gm)			
Range	2.9-21.2		0-85
Mean ±SD	8.4±3.4	25	32.8±14.4
Median (IQR)	7.8(6-10.2)		31(23.9-40.7)

 Table 5: Actual intake, DRI and % DRI of water and fibers among studied Down syndrome children (N=106)

SD= Standard Deviation IQR=Interquartile Range

Table (6): show that Actual intake more than, DRI and % DRI of vitamins was taken in excess from vitamins (A, C, Thiamine, and Riboflavin).

Variables	Intake	DRI	% DRI
Vitamin A			
Range	17.2-8470.1	400-900	2.8-2021
Mean ±SD	1062.9 ± 2059.6	631.1±199.6	173.3±352.1
Median (IQR)	276.9(165.9-470.4)	600(400-900)	44.5(30.4-86.8)
Vitamin C			
Range	0-362.0	25-75	0-1033.4
Mean ±SD	79.7±64.7	48.1±19.9	200±200.1
Median (IQR)	56.0(32.3-120.3)	45(25-75)	123(63-273.2)
Thiamine			
Range	0.24-3.5	0.6-1.2	25.8-418.3
Mean ±SD	$0.99{\pm}0.5$	$0.8{\pm}0.2$	115 ± 60.4
Median (IQR)	0.89(0.67-1.11)	0.9(0.6-1.2)	100(76.4-148.7)
Riboflavin			
Range	0.28-42.3	0.6-1.3	31-4700
Mean ±SD	2.51±6.2	$0.9{\pm}0.3$	305.5±745.7
Median (IQR)	0.92(0.68-1.3)	0.9(0.6-1.3)	105.5(73.4-173.8)
SD- Standard Deviation	n IOP-Interquartile Pange		

SD= Standard Deviation IOR=Interguartile Range

4. Discussion

Recording Anthropometric parameters, in the present study, there is a non-statistically significant difference between anthropometric measures of studied DS children according to gender. Height for age in children with DS showed that more children (79.2%) were normal, and (17.0%) were stunted, while fewer children (3.8%) were tall. This result is in line with previous research reporting most of the participants (87.5%) attained normal heights in comparison with the special growth charts developed for children with DS. Only (6.2%) participants were short for their age, and (6.3) children were tall (Osaili et al., 2019). Weight for age was significantly increased in (13.2%) of subjects, while decreased in (2.8 %) which were under weight. More children (84.0 %) were had normal weight. Our results are in agreement with the results of Osaili et al. (2019) found that more children with DS had normal weight (71.7%), while underweighted children were (7.7%) and over weighted children were (10.3%). However, AbdAllah et al. (2013) reported that Weight for age was significantly decreased in participant with Down syndrome compared to the controls in both sexes and particularly in the younger age group.

Head circumference for age was presented in table (2). It was found that, there is a nonstatistically significant differences between male and female, which were had normal head circumference (76.5 % and 78.6 %, respectively). While (6.3 % and 2, 4 %, respectively) male and female had small head circumference. Also, (17.2 % and 19 % respectively) male and female had large head circumference. This result agrees with Panneer Selvi et al. (2017) showed that (63%) of Down syndrome cases having normal range of head circumference that have been calculated using CDC and WHO chart.

This study showed the mean value of BMI (24.9 ± 9.6). These results are supported by Zemal *et* al. (2015) who reported that BMI values resulted normal.

Our study show there is statistically significant correlation (r=0.457, p 0.008) regarding relation between dietary habits score of DS children and socioeconomic status statistically as recorded by Wanjihia et al. (2021) found that there was also significant correlation between knowledge score and the wealth quintile or Socio-Economic Status (SES) (r=0.681, p=0.012). In this study, a factor such as household income may have had an impact on nutritional status not withstanding mother's nutrition knowledge as households with better income may have had a higher potential of providing appropriate complementary foods as compared with households of low income.

This study shows non- significant correlation (r=0. 159, p 0. 104) regarding the correlation between awareness score of caregivers of DS children and dietary habits score. These results are in line with Wanjihia et al. (2021) reported there was non-significant relationship between maternal nutrition knowledge and feeding practices with the nutritional status. However, Jemide et al. (2016) showed a significant relationship between maternal nutrition knowledge and feeding practices with the nutritional status of children.

Food intake as assessed using food frequency reflected a high intake of carbohydrate rich foods. There was low consumption of fruit and vegetables among children despite its presence in the nearby market and its low cost, highlighting the lack of awareness as one of the causes. Foods from animal sources except for milk were consumed less frequently despite this being a pastoralist community. Lack of adequate maternal education and nutritional knowledge among caregivers has been cited as a potential explanation for unhealthy dietary habits for the children. Nutritional knowledge of caregivers is a determinant of the type and quality of diet provided to the children which affects the nutrition status.

The present study shows that there was non-significant difference in actual intake between males and females. Actual intake of Caloric and all macronutrients increases DRI at high rate. This increase is (1.77, 2.08, 1.77) times of DRI for calorie, protein, carbohydrate, and fat, (91%,74%,78.3%,94.3%) DS Pupils respectively. These results agree with Magenis *et al.* (2018) who found that regarding energy (68.4%) of DS children were above recommendations, and (21.0%) of DS children were below recommendations. According to the Acceptable Macronutrient Distribution Range (AMDR), (94.7%) of DS participants were above protein intake recommendations; (78.9%) of DS group were above carbohydrate recommendations. In addition, (84.2%) of DS group were above lipids recommendations. Because high percentage consumes (black bread, white bread and rice) were (68.2%,71.0%,59.8%) respectively once a day, a high percentage eat pasta from (2-3) times a week, / day. Also show that a high percentage more than half is eating of meat group. It also shows that a high percentages like eating (fried fish &grilled fish) once a week (41.1%,40.2%) respectively while (seed oil, hydrogenated ghee and Egyptian ghee) are used extensively daily (86.9%,57.9%54.7%) respectively while (0.0) none use.

Our study show that actual intake more than, DRI and % DRI was taken in excess from Water (167.5 \pm 45.6) while Actual intake less than, DRI and % DRI from Fibers (32.8 \pm 14.4). In harmony with these findings Magenis *et al.* (2018) reported that 57.9% DS participants were below fiber recommendations Approximately, 60% of DS participants had low fibre intake. This fact coupled with a high rate of insufficient water intake may give rise to constipation and slow intestinal peristalsis.

The current study shows a remarkable increase in the actual intake of micronutrients (100%) children with DS excess intake from sodium, (83%) from potassium, (93.4%) iron, (75.5%) from zinc and (88.7%) copper, while remarkable decrease in the actual intake of calcium and magnesium compared to the DRI. This is in line with previous finding by Skrzypek et al. (2021) who found the actual intake of sodium is 7 times, while the actual intake of calcium and magnesium is about half when compared to RDI% sodium intake in DS individuals, a high percentage of DS children and adolescents exceeding the recommended values (89.5%); in children and adolescents with DS, while Calcium intake was too low inrelation to the EAR norm in as many as 12 subjects (66.7%). Because also, it shows that DS eat full-fat cheese (salty cheese) and cooked cheese, were consumed by a large number of subjects daily (once /day), (52.8% and 31.1%), while the use of Quraish cheese was (13.2%) once /day.show that (8.5%)suffered from Bread and milk allergy while(91.5%) did not suffer . Ghazzawi et al. (2022) no differences between both gender among mineral intakes. However, magnesium, potassium, zinc, copper, manganese and selenium showed lower intake when compared to the RDA Zinc deficiency is one factor that may influence the growth and development of children with DS since this nutrient plays an important role in child development and growth. According to a study zinc supplementation in children with mild deficiency increases appetite, growth velocity and GH, somatomedin and IGF-1 levels and improves immunity.

The current study shows a remarkable decrease in the actual intake of calcium and magnesium compared to the RDI (95.3%) DS take less than %DRI of ca. a small study found that children with Down syndrome tended to consume more calcium than the recommended daily allowance (Savage *et al.*, 2007; Mazurek and Wyka, 2015) (Zinc, selenium and calcium deficiencies are ma- nifest in DS children. The former signifi- cantly affects thyroid metabolism, immunity, ensuring appropriate stature, nucleic acid metabolism, and gene expression and is a component of many enzymes. Zinc deficiency causes abnormal body growth, lowered immunity and thyroid dysregulation (mainly hypo- thyroidism).

The present study shows that actual intake more than, DRI and % DRI of vitamins was taken in excess from vitamins (54.7%) from vit C, (49.1 %) from Thiamine, and (51.9%) from Riboflavin). These results are supported by Skrzypek *et al.* (2021) revealed that the intake of vitamins B1, B2, and B6 was excessive in all respondents and exceeded (110%) of the Estimated Average Requirement EAR Vitamin C intake corresponded to the (EAR) norm in one case, while excessive intake was observed in the remaining subjects, with the greatest excess of the norm up to(706.5%) of EAR. dis agree with

Ghazzawi *et al.* (2022) reported that vitamin and mineral intakes were lower overall in subjects with Down syndrome than in the RDA, except for vitamin B3, B12 and D. This finding may be related to the feeding difficulties in a patient with Down syndrome making them unable to consume adequately the fresh natural sources. For Vitamin C, Down syndrome individuals significantly consumed more Vitamin C than the recommended daily allowance.

The present study shows that actual intake less than, DRI and % DRI of vitamin A(80.2%) subjects with Down syndrome takeless than %DRI of vitA Ghazzawi *et al.* (2022) reported that "Serum Vitamin A levels have been reported to be below in individuals with Down syndrome" this is possibly due to malabsorption.

The present study shows that actual intake less than, DRI and % DRI of vitamin C (44.3%) subjects with Down syndrome takeless than %DRI of vitA Ghazzawi *et al.* (2022) reported that For Vitamin C, Down syndrome individuals significantly consumed more Vitamin C than the recommended daily allowance, Samarkandy *et al.*, (2012) many children with Down syndrome had a deficiency of Vitamin C according to serum tests which correlated to dietary intake.

5. Conclusion

The socioeconomic status of the caregivers had a positive correlation with the knowledge score of the caregivers. Most of the caregivers did not have any nutritional knowledge which was assessed as knowledge of food groups and sources and functions of different foods.

Recommendations

This study recommends continued communication of nutrition messages to caregivers as a strategy towards behavior change on dietary practices.

Conflict of interest

The authors reported no conflict of interest.

Acknowledgement

Authors would like to thank Dr. Dr. Heba El-Sayed Daoud Faculty of Medicine Genetics department at Tanta University who shared in the study, pupils and their parents, caregivers at the Intellectual Education Schools and also the administration of institutions where the study was carried out for their cooperation

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