Evaluation of Retinol level among Preschool Children, Pregnant and Lactating women in Baghdad

By

Nutrition Researches Institute

Supervised by

Dr.Alaa Sha'lan Hussien
M.B.Ch.B.,M.Sc.C.M.
NRI Director

Dr.Saad alddin Hussien Ali
M.B.Ch.B.,F.I.B.M.S.C.M.
NRI Deputy Director

Prepared by

Sawsan Mohammed Ali D.CH

list of Contents

List of abbreviations………………………….…………….………….…..…………….. 3

Summery ………………………………………………….….……..……………… 4

Introduction ……………………..……………….…………….……….……………… 5

Aims of the study …………………………..…………….……….…………………. 6

Materials and Methods ……………………………………….….….……………..…. 6

Results and discussion ………………………………….….………………..….… 10

Conclusions …………………………..…………….…………………...……... 26

Recommendations ………………………….…………………………..…..... 27

References ………………………………….………………..…………...…… 28

List of Abbreviations

CI ………………………..Confidence Interval

CFSVA …………………..Comprehensive Food Security and Vulnerability Analysis

df ………………………...Degree of Freedom

Hb ………………………..Hemoglobin

HPLC ……………………High Performance Liquid Chromatography

P …………………………Probability

r …………………………correlation coefficient

SD ………………………Standard Deviation

SR ………………………. Serum Retinol

VAD ……………………..Vitamin A Deficiency

WHO……………………World Health Organization

BMI………………………Body Mass Index

Summery

The present study is an attempt to estimate the prevalence of vitamin A deficiency among preschool children, pregnant and lactating women attending primary health care centers in Baghdad, in addition to figure out the relation between vitamin A deficiency with some demographical, clinical, biological, and environmental variables. The study was conducted during the period from October to December 2009.

The sample was comprised of 490 subjects, (198) (40.4%) Lactating women, (164) (33.5 %) pregnant women and a total of (128) (26.1 %) under 6 years old children attending ten primary health care centers in Baghdad.

The data were collected through direct interview; blood samples were taken and analyze for serum retinol (SR) and hemoglobin (Hb) level, anthropometric measurement were obtained for the study sample.

The study showed that the prevalence of vitamin A deficiency in preschool aged children (below 6 years) was (38.3 %); and that for lactating women and pregnant women were (7.1 %) and (25 %) respectively.

Forty percent of pregnant women, (25.8 %) of lactating women and a total of (58.6 %) preschool children were anemic, A correlation coefficient between SR and Hb concentrations was significant (N=490, r=0.533, P<0.0001).

Introduction

Vitamin A (retinol) is a fat –soluble substance stored in the body organs, principally the liver. It released into blood stream, becoming available use by cells throughout the body, including those of the eye [1]. It is one of most important nutrients which are essential to all, especially children and pregnant women [2, 3] and plays an important role in cellular differentiation, which is critical in growth, reproduction and immune response. Children with vitamin A deficiency (VAD) have a tendency to be more affected by infection [4, 5]. In addition, VAD is associated with increased mortality in young children [6, 7].

Vitamin A deficiency is the most common cause of childhood blindness, causing 250000 – 500 000 children to go blind every year, half of whom will die within the year. Approximately another 150 million are at increased risk of dying in childhood from infectious diseases owing to inadequate vitamin A status, the World Health organization (WHO) estimated that globally nearly 14 million children affected annually by clinical vitamin A deficiency (xerophthalmia) and 190 million were at risk of subclinical vitamin A deficiency [8].

Vitamin A deficiency is essentially attributed to inadequate dietary intake. The episodic consumption of food sources of preformed vitamin A such as dairy products, liver, eggs and cereal rich alimentation characterize the most affected countries [9, 10]. In developing countries, blindness, measles and severe diarrhea are related to VAD [11,12].When maternal vitamin A status is low, breastfed infants are likely to become deficient [13].

Nutritional surveys have shown a close relationship between vitamin A and iron metabolic indicators, and vitamin A is considered to influence anemia by modulating erythropoiesis and iron metabolism and enhancing immunity to infectious diseases.

Nutritional surveys in several countries have shown an inadequate intake of some micronutrients. A possibility to challenge this situation is the fortification of selected foods with micronutrients [14,15].

Aims of the study

1) To estimate the prevalence of vitamin A deficiency among under 6 years old children , pregnant and lactating women in Baghdad .
2) To figure out the relation between vitamin A deficiency with some demographical, clinical, biological, environmental variables.

Materials and Methods

Place

The study was carried out in10 primary health care centers in Al-Karkh and Al-Rusafah sectors in Baghdad.

Time

The study was conducted during the period from October to December 2009. Each center was visited for 2-3 hours /day, four days / week for two month during the study period in order to obtain the required sample size, which was 490 subjects, (198) Lactating, (164) pregnant and a total of (128) under 6 years old children were included conveniently in this cross-sectional study.

Methods

The data were collected through direct interview; blood samples were taken and analyze for serum retinol (SR) and hemoglobin (Hb) level, anthropometric measurement (height and weight) were obtained for all participants. Weight was measured to the nearest 0.1 kg with a battery-powered digital scale (Uniscale). Height was measured to the nearest 0.1 cm with a height board. Height was measured in a standing position (and length is taken for children less than 2 years old). Calculate Z-scores for height-for-age, weight-for-height, and weight-for-age. A cut-off of less than minus two standard deviations (-2SD) was used to define stunt (height/length-for-age Z-score), wasting (weight-for-height/length Z-score), and underweight (weight-for-age Z-score).

Blood sampling and biochemical measurements

A blood sample (5 ml) was collected by vein-puncture, and the volume of sample was about 1 ml for children whose ages were less than 1 year old. The specimens were collected in EDTA tubes, measure hemoglobin by hemiglobincyanide, continuously shielded from light, centrifugation. Aliquots of plasma were made and immediately frozen at -20°C; the plasma retinol concentration was determined by reversed-phase high-performance liquid chromatography (HPLC). A total of 0.2 ml plasma was added to 0.2 ml standard solution of retinyl acetate, and was depolarized by ethanol. Plasma vitamin A was extracted with hexane (1 ml) and a portion of the sample (0.5 ml) evaporated to dryness under nitrogen and re-dissolved in 0.5 ml mobile phase(methanol: water, 95:5 by vol.), and injected into a C_{18}, reversed phase HPLC column (5 mm particle size; knauer Instruments, Inc.). The flow rate of mobile phase was 2.5 ml/min. Retinol was detected by monitoring the absorption at 287 nm in a knauer ultraviolet detector. The vitamin A concentration was quantified to the peak high of the internal retinyl acetate standard. For plasma retinol, the within-assay and between assay CVs were 5% and 7%, respectively.

The following figures describe the process of determining plasma retinol levels during which Injection of 20 µl of human plasma extract and retinyl acetate as internal standard. The retinol had a retention time of 2.4minutes and 3.1 minutes for the internal standard. All compounds are resolute in relation to the peak of retinol as in figure (1 and 2).

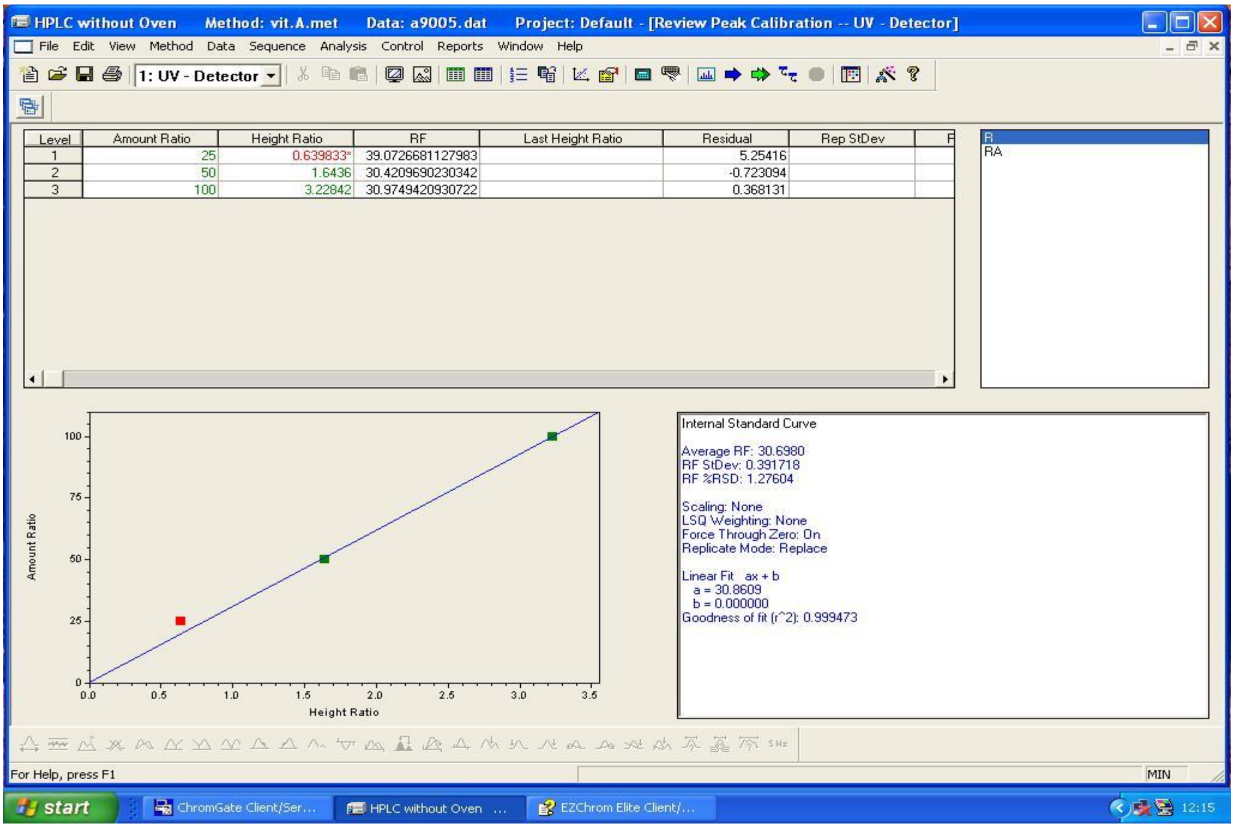


Figure (1): Typical chromatograms of calibrators as detected with Calibration mixture containing 50 µg/dl internal standard, Linearity fit, Concentration (5–100) µg/dl, r (0.999)

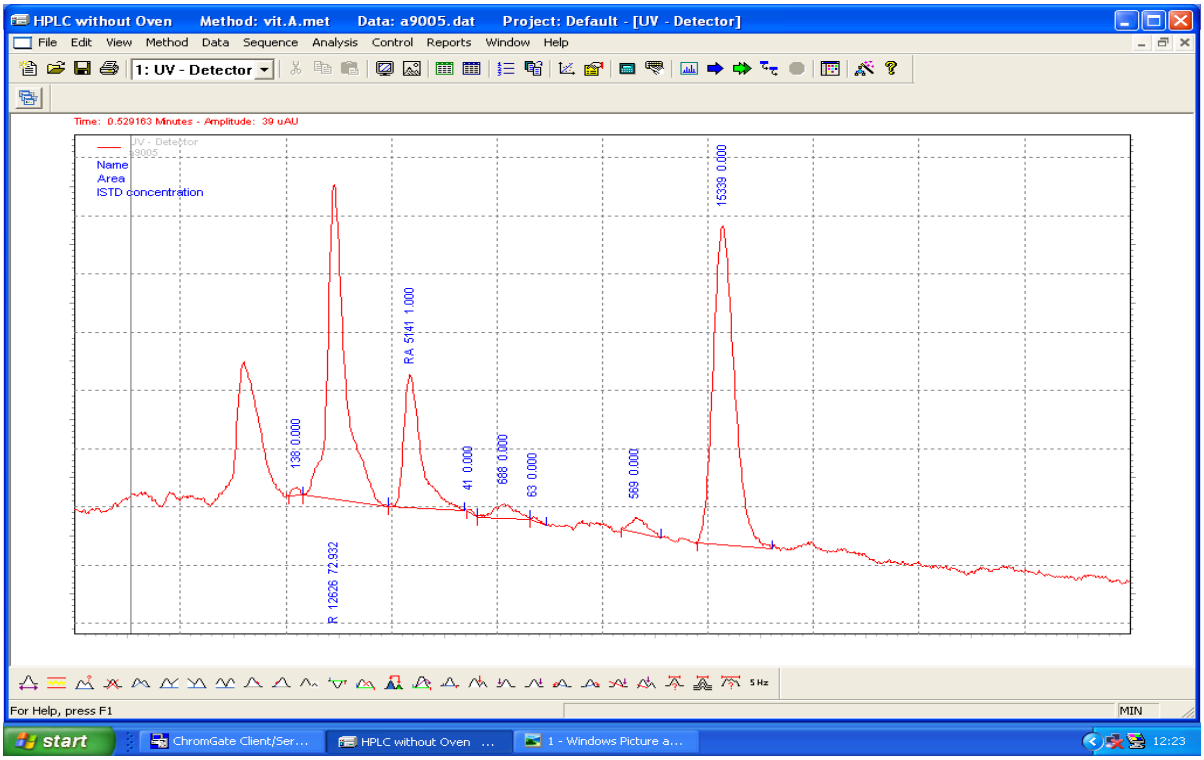


Figure (2): Chromatogram of human plasma analyzed by HPLC

Definition of outcome

The prevalence of anemia was determined according to the WHO criteria, i.e., hemoglobin <11 g/dl for to under 6 years old children and pregnant women, hemoglobin <12 g/dl for lactating women. According to the WHO criteria, a serum (plasma) retinol concentration <0.7 μmol/l (< 20 μg/dl) was classified as VAD, values 0.70-1.05 μmol/l (between 20 and 30 μg/dl$) were taken as marginal VAD [8].

Statistical Analyses

Data analysis for the inquired variables was done by using data analysis soft wares namely (SPSS and Excel) and was represented by using tables and charts describing the distributions of variables under study according to demographic, biologic and environmental factors reaching to determining the answer for our main research question in the study.

Results and Discussions

The study sample comprised 490 subjects, 198 (40.4%) Lactating women, 164 (33.5 %) pregnant women and a total of 128 (26.1 %) of under 6 years old children (been classified into 3 groups (below 6 months (4.7 %) , 6 months to below 2years (22.6 %) and 2 year to 6 years (72.7%)) as shown in Table (1).

Table (1): The distribution of study sample by age category

Age Category		Frequency	Percent
Under 6 years	Below 6 months	6	4.7%
	6m - <2Y	29	22.6%
	2y - 6y	93	72.7%
	Total	**128**	**26.1 %**
Lactating		**198**	**40.4 %**
pregnant		**164**	**33.5 %**
Grand Total		**490**	**100.0 %**

Eighty –three percent (407 subjects) of the overall study subjects are from urban areas which also represents the highest proportions in pregnant and lactating women (78.7%, 83.3 %) respectively and preschool children (88.3%) as shown in figure (3) and annex (1).

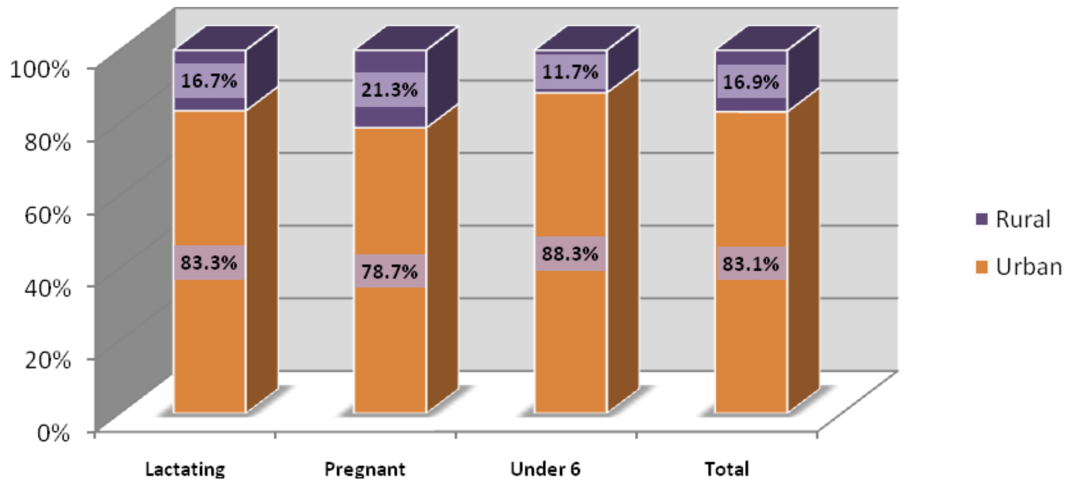


Figure (3): Distribution of study groups enrolled by living environment

The highest percentage of study subjects were from Al-Rusafah health directorate (73.8%) , in which 7 out of 10 primary health centers were included as shown in figure (4).

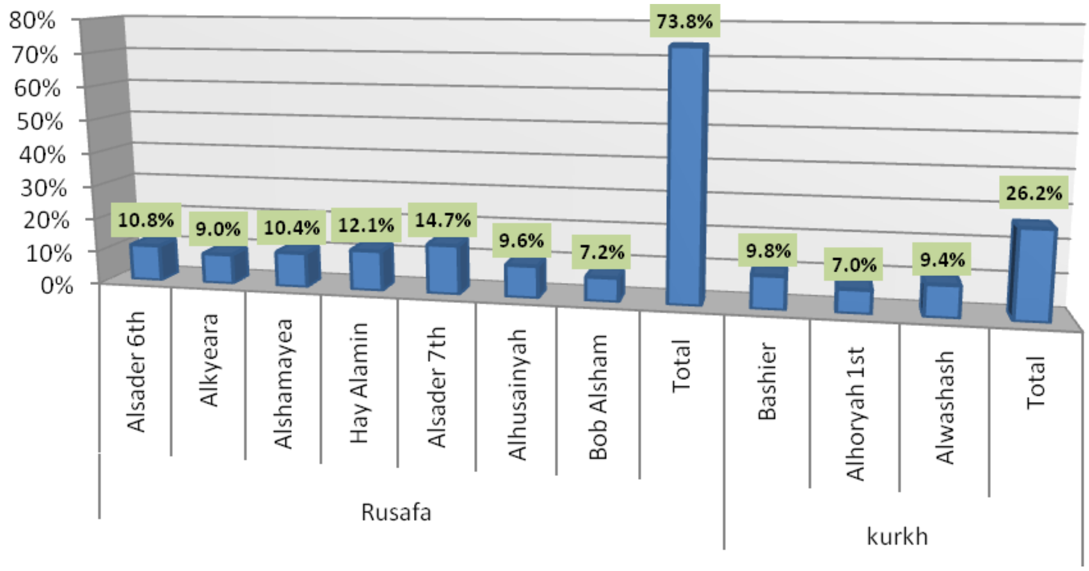


Figure (4): Distribution of study subjects by Sectors

The mean body weight and the mean Length/height are distributed according to age categories used in the study as shown in Table (2)

Table (2): Mean anthropometrical measurements (weight and height) distributed according to study groups

Age category	Anthropometry	
	Weight (mean ± SD)	Height (mean ± SD)
Below 6 years	14.37 ± 6.66	91.63 ± 14.21
Pregnant	70.09 ± 16.83	157.69 ± 11.39
Lactating	70.72 ± 13.14	157.69 ± 5.52
Total	56.01 ± 27.89	140.7 ± 30.71

Data obtained from the above anthropometrical measurements of (128) preschool children and by the use of WHO growth reference tables , results revealed that the overall distribution of malnutrition indicators prevalence (wasting, stunting and underweight) shown in figure (5), compared to the national average (CFSVA, 2008) were all lower than national average (2.5 % , 7.7 % , 4.3 %) respectively which only can give a clue about malnutrition rates in related areas , and a larger sample size is needed to reflect the results on the general population nation-wide.

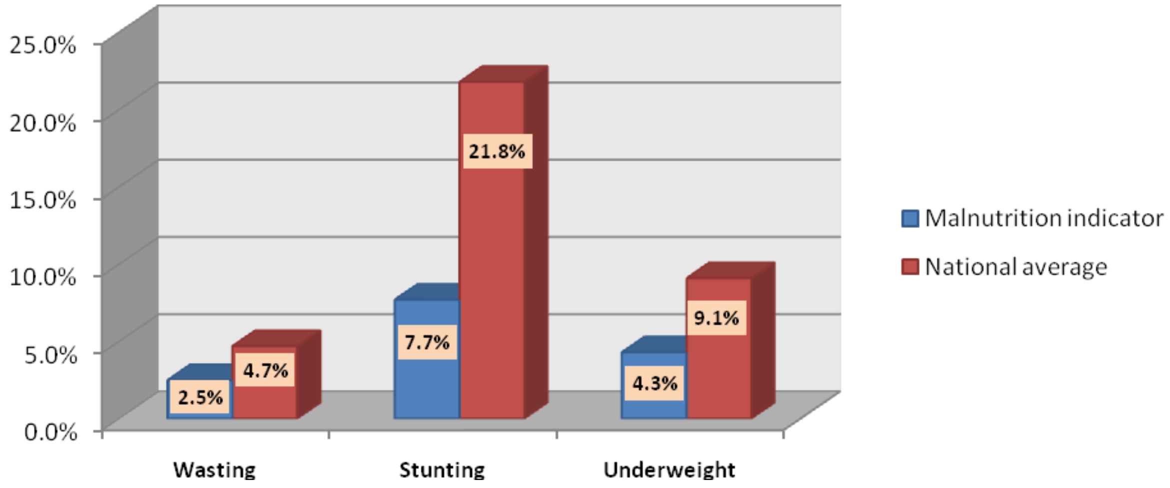


Figure (5): Malnutrition indicators compared to the national averages

Different dietary, pathological and biological parameters were included in this study and figure (6) ;annex (2) show the frequency distribution of these factors in the preschool children; most of them (57.5 %) had diarrhea during the last month,(65.4 %) suffered from respiratory tract infection ,(92.9 %) had measles,(79.4 %) suffered from other infections (Mostly tonsillitis and bronchitis (45% , 20 %) respectively) , Visual disturbances and night blindness were seen in (88.1 %) of these children , (80.8 %) stated that they had vitamin supplements during the last month,(68.3 %) drink tea (about (66.7 %) were drinking tea during a meal).

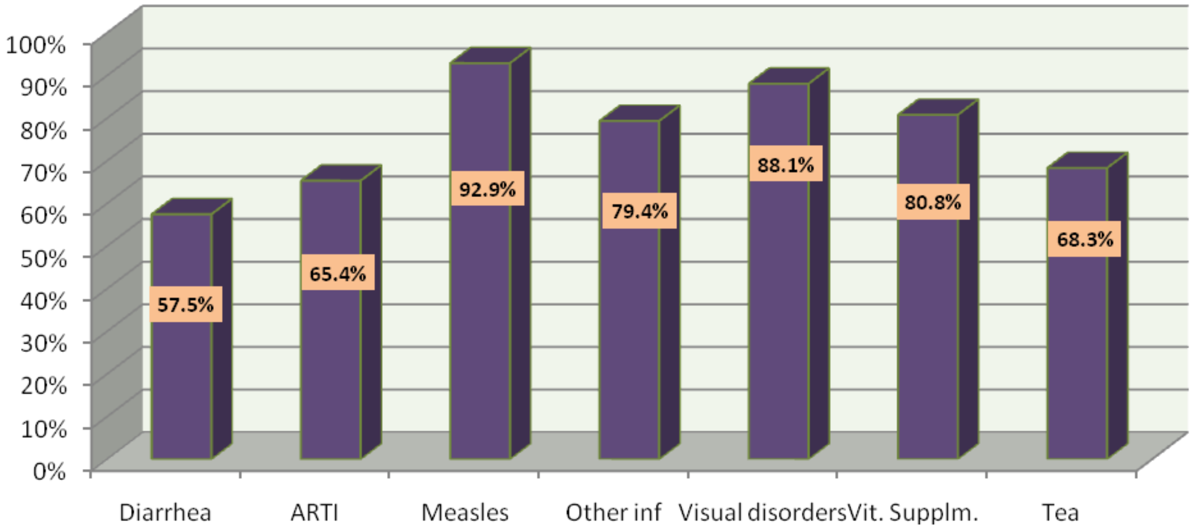


Figure (6): distribution of pathological and biological parameters included in the study

Most of the food groups included in the study questionnaire were consumed in a three or more times per week consumption pattern and this was true in the three different study groups (below 6 years old children, pregnant and lactating women) as shown in figure (7) which has to be linked to construction of Food Consumption Score cut-off for best match of proportion of food pattern.

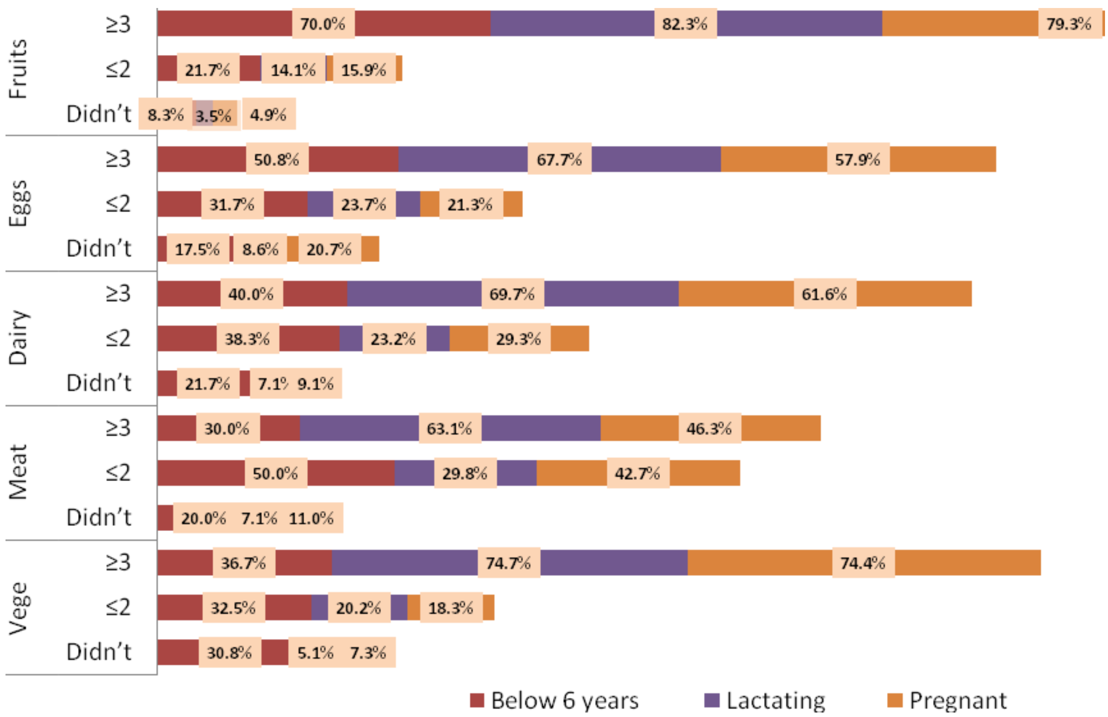


Figure (7): Frequency distribution of food groups consumption pattern according to study groups enrolled

Eighty – five percent of lactating women reported not taking any vitamin A supplements during the last month (at the study time) , (66.8 %) of women did not have any Ferro folic supplements during the last month, (78.7 %) did not have any visual disturbances or night blindness, (82.9 %) of pregnant and lactating women enrolled in the study drink tea and most of them (71 %) have it during a meal.

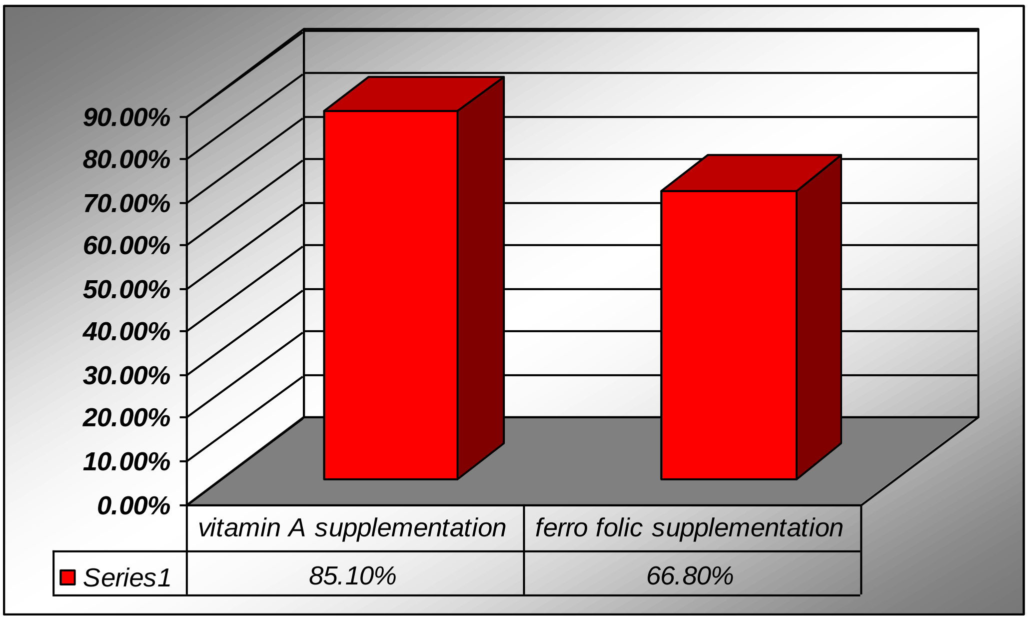


Figure (8): Vitamin A and Ferro folic supplementation

The present study showed a percentage of vitamin A deficiency (38.3 %) among preschool aged children (below 6 years) which is increasing with age with a strong significant relationship ($p<0.0001$) , (7.1 %) among lactating women and (25%) among pregnant women; which can be defined as a corroborative evidence of a public health problem in preschool aged children and pregnant women (more than 15 % of under-5-years-olds with serum retinol below 20 µg/dl) [(8)] as shown in figure (9) The overall mean (±SD) serum retinol level in this study is (30.4±12.6) ug/dl, in preschool children this mean (±SD) is (24.9±10.6) ug/dl and (54 %) of them were female, whereas in pregnant and lactating women the mean serum retinol level (±SD) is (27.1±9) ;(36.8±12) ug/dl respectively

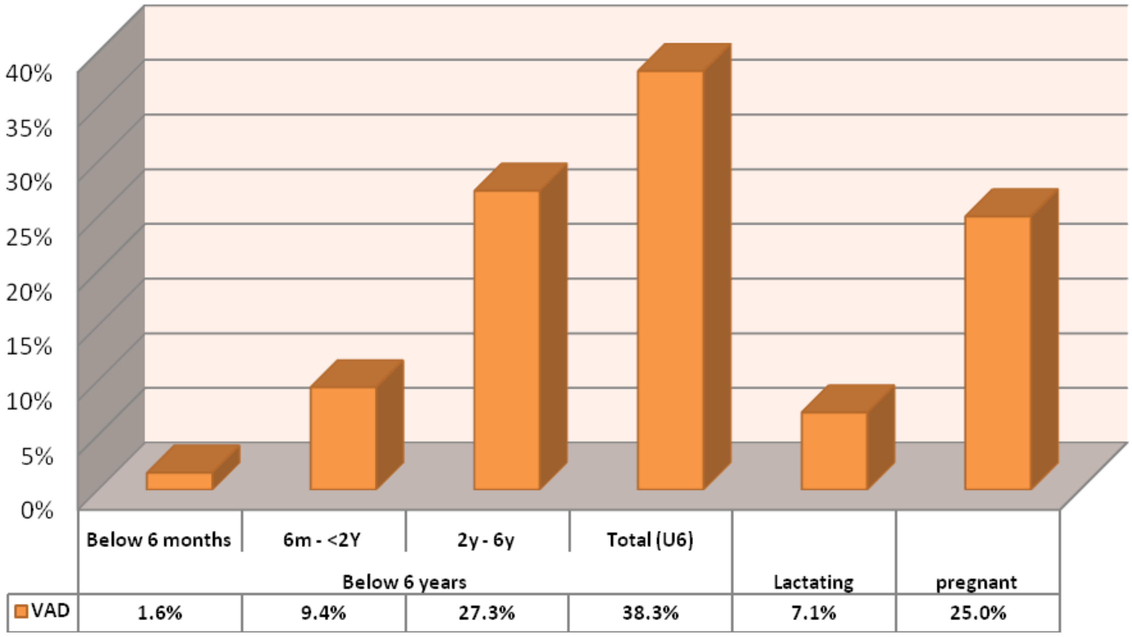


Figure (9): Prevalence of VAD (SR<20 ug/dL) by study groups

Most of VAD cases were found in Alrusafah districts (68.3%); mainly in Alshamaiyah, Alsader (7^{th}), Alkayarah and Hay alamin health centers (17.3%, 13.5%, 13.5%, 12.5%) respectively as shown in figure (10).

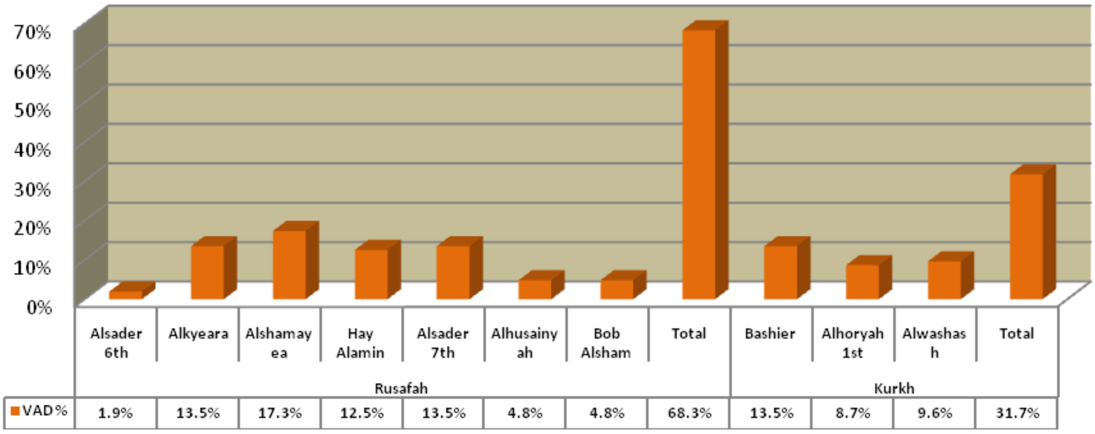


Figure (10): Prevalence of VAD by health centers

Anemia status was estimated in this study by determining Hb level, the mean Hb level (±SD) in preschool children is (11 ±1) g/dl, while the mean Hb level in pregnant women is (11.4±1) g/dl, as for lactating women, Hb level is (12.1±1) g/dl, while (40.8 %) of pregnant women are anemic, (25.8 %) of lactating women are anemic and a total of (58.6 %) of included preschool children were anemic with a strong statistical significant differences between the age category and anemia (P<0.0001) as shown in table (3).

Table (3): The prevalence of anemia among study groups

			Anemia Status		Total
			Anemic	Not Anemic	
Below 6 years	Below 6 months	Count	2	4	6
		% of Total	**33.3 %**	66.7 %	4.7 %
	6m - <2Y	Count	23	6	29
		% of Total	**79.3 %**	20.7 %	22.6 %
	2y - 6y	Count	50	43	93
		% of Total	**53.8 %**	46.2 %	72.7 %
	TOTAL	Count	75	53	128
		% of Total	**58.6 %**	41.4 %	26.1 %
Lactating		Count	51	147	198
		% of Total	**25.8 %**	74.2%	40.4 %
pregnant		Count	67	97	164
		% of Total	**40.8 %**	59.2 %	33.5 %
Total		Count	193	297	490
		% of Total	**39.4 %**	60.6 %	100 %

The overall proportion of subjects who had co-occurrence of VAD and anemia was (42 %) ((81out of 193) anemic cases included in the study). A close association between vitamin A deficiency and anemia has been shown in many nutritional surveys from around the world, and perhaps this is not surprising, given the widespread prevalence of nutritional anemia and vitamin A deficiency in developing countries[16]]. Most of these epidemiological surveys did not identify the underlying causes of anemia, and often the proportion of subjects with concurrent vitamin A deficiency and anemia are not stated. The surveys generally demonstrate that there is often a high prevalence of vitamin A deficiency and anemia in the same population. In the nutrition survey from Paraguay, hemoglobin and plasma retinol concentrations were highly correlated, with a correlation coefficient of 0.90 (Interdepartmental Committee on Nutrition for National Defense 1998). Pooled data from surveys conducted in Vietnam, Chile, Brazil, Uruguay, Ecuador, Venezuela, Guatemala and Ethiopia showed a high correlation (r=0.77, P<0.0001) between hemoglobin and plasma retinol concentrations [17, 18, 19]

A correlation between hemoglobin (Hb) and plasma or serum retinol (SR) concentrations has been described in many studies, including studies of preschool children from Pakistan (r=0.367,P<0.0001); [20], school-aged children in Central America (r=0.209, P<0.05) [21] , school-aged children from Bangladesh (r=0.31, P<0.001;), adolescent girls in Malawi (r=0.161,P<0.08); [22].

In this study, the serum retinol concentrations have been scattered against Hb level in 3 scatter diagrams representing the correlation between these 2 parameters and Figures (11, 12, and 13) show the serum retinol and Hb concentrations among the three study groups.

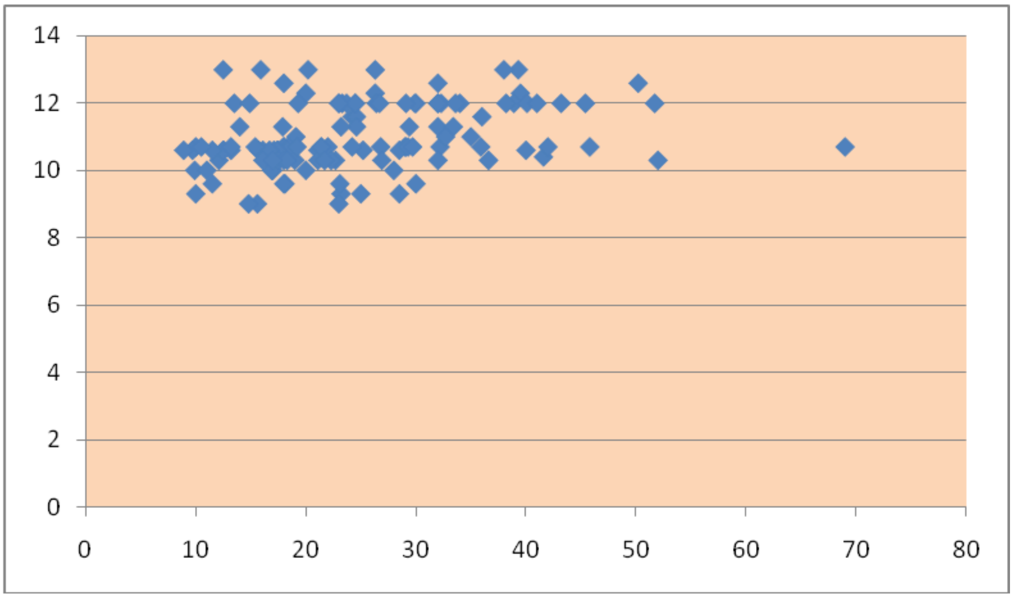


Figure (11): A scatter diagram of serum retinol levels (ug/dl) (X-axis) and Hb levels (gm/dl) (Y-Axes) among preschool children

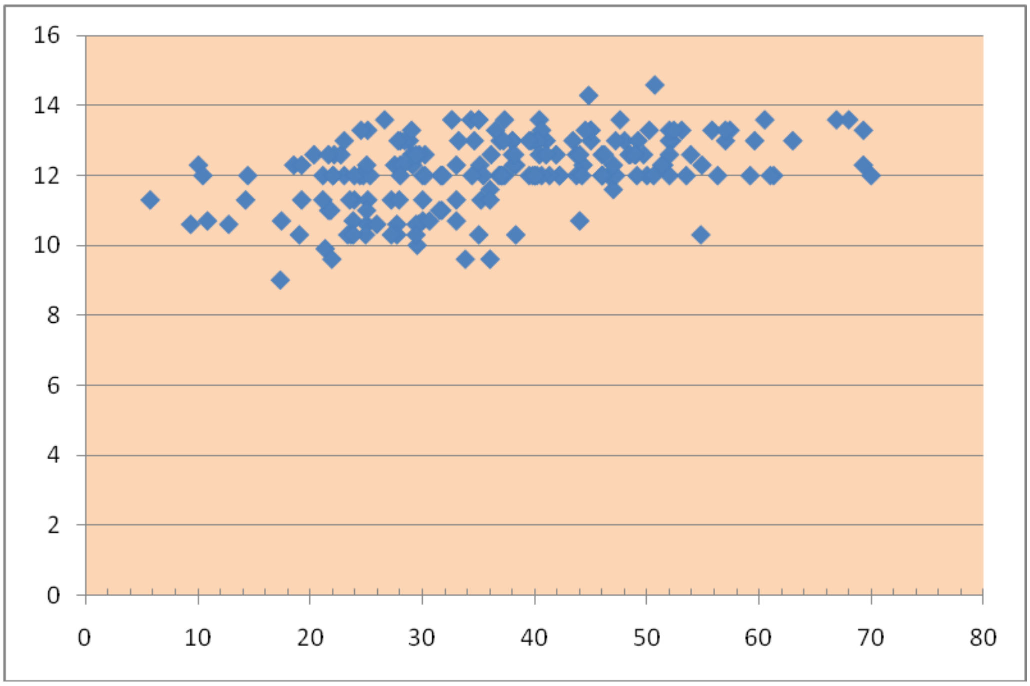


Figure (12): A scatter diagram of serum retinol levels (ug/dl) (X-axis) and Hb levels (gm/dl) (Y-Axes) among Lactating women.

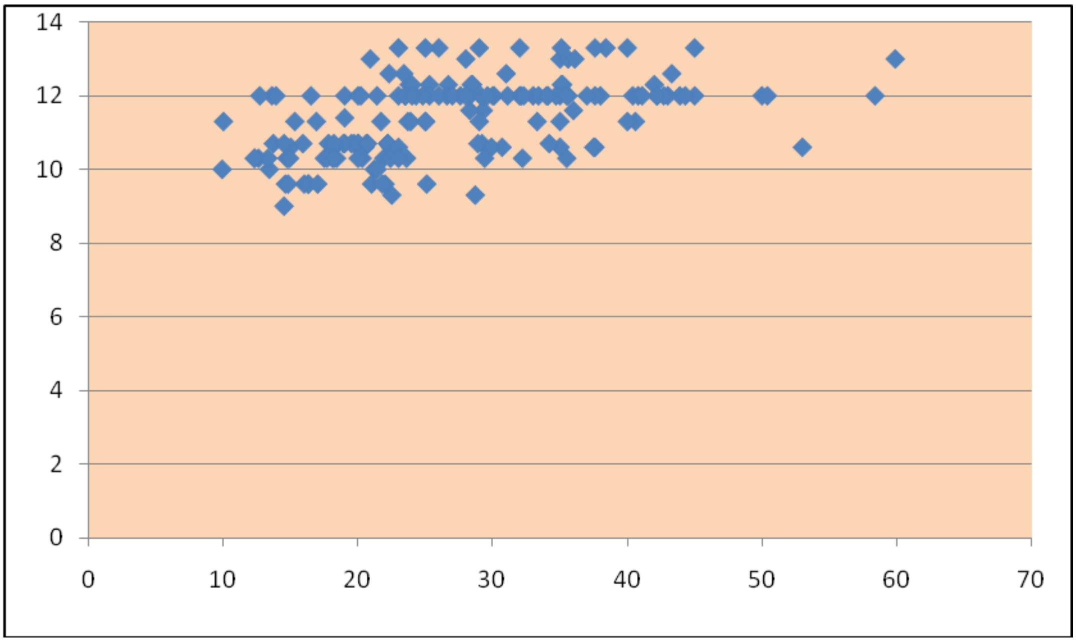


Figure (13): A scatter diagram of serum retinol levels (ug/dl) (X-axis) and Hb levels (gm/dl) (Y-Axes) among pregnant women.

A correlation can be established between the overall serum retinol (SR) concentrations and Hb levels using the Bivariate Correlations using Pearson's correlation coefficient (r) which is a measure of linear association. Two variables can be perfectly related, but if the relationship is not linear, Pearson's correlation coefficient is not an appropriate statistic for measuring their association, A correlation coefficient of 1 means a perfect correlation while a zero means there is no correlation between the 2 factors understudy, Therefore in this study A correlation coefficient between the previously mentioned measures was significant (N=490, r=0.533, P<0.0001).

This was true also in correlating SR and Hb concentrations among the three study groups (Pearson Correlation is significant at the 0.01 level) and shaded areas in table (4) represent these strong correlations between the 2 levels and their p-values (P<0.0001).

Table (4): Correlation between serum retinol and Hemoglobin levels among study groups.

		SR preschool	Hb Preschool	SR pregnant	Hb pregnant	SR lactating	Hb Lactating
SR preschool	Pearson Correlation	1					
	Sig. (2-tailed)						
	N	126					
Hb Preschool	Pearson Correlation	.322	1				
	Sig. (2-tailed)	.000					
	N	126	126				
SR pregnant	Pearson Correlation	.089	.141	1			
	Sig. (2-tailed)	.324	.116				
	N	126	126	166			
Hb pregnant	Pearson Correlation	.024	-.011	.508	1		
	Sig. (2-tailed)	.789	.900	.000			
	N	126	126	166	166		
SR lactating	Pearson Correlation	-.017	-.104	-.097	.020	1	
	Sig. (2-tailed)	.846	.247	.215	.797		
	N	126	126	166	166	198	
Hb Lactating	Pearson Correlation	-.090	-.052	-.044	-.058	.453	1
	Sig. (2-tailed)	.314	.560	.570	.460	.000	
	N	126	126	166	166	198	198

The evidence that vitamin A deficiency causes anemia through modulation of iron metabolism is strong and supported by observations from both experimental animal models and human studies. The hypothesis that vitamin A deficiency contributes to anemia through depressed immunity to infection and an increase in the anemia of chronic disease is reasonable, but there is a paucity of data to support this idea directly. Most of the controlled clinical trials of disease-targeted vitamin A supplementation have not examined anemia as an outcome measure and, in retrospect, measurement of hemoglobin, hematocrit and iron status indicators would have been fairly easy to include.

Distributing VAD by frequencies of food group consumption shows that the least percentages of VAD were found in all food group frequency consumption patterns as shown in figure (14) among all study groups with only a statistical significant association between frequencies of consuming meat and serum retinol concentration with a chi square (χ^2) value =9.92 for a df=2 and a P=0.007 (p<0.05) with differences between observed and expected values in pregnant and lactating women.

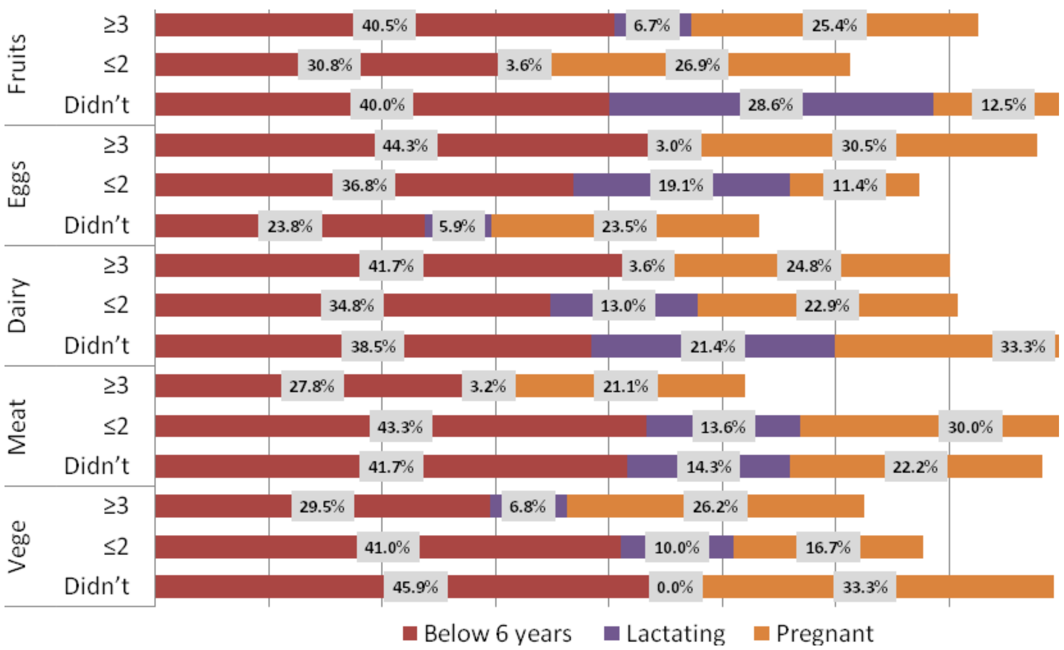
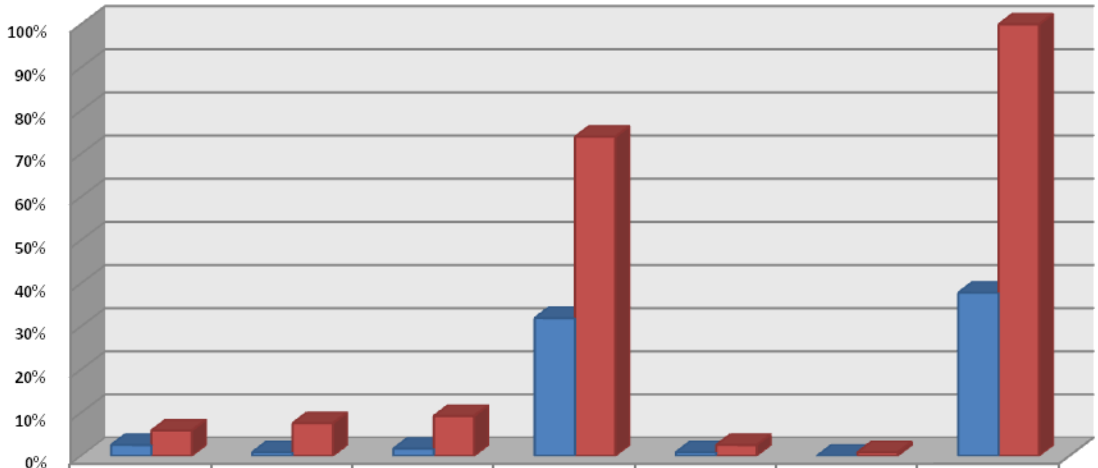


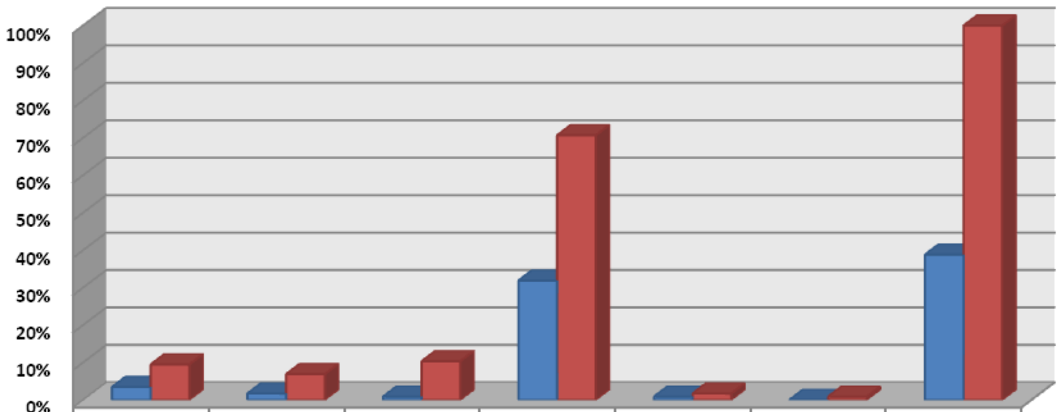
Figure (14): Proportion of VAD with frequency of food group consumed among study groups

Proportions of VAD cases were distributed among growth indicators categories as shown in figures (15, 16, 17, 18); annexes (4, 5, 6, 7) which give you an idea about the prevalence of VAD in relation to growth indicators but not conclusive in this study having a non-statistical significant relationship between different SR means in each category (P>0.05).



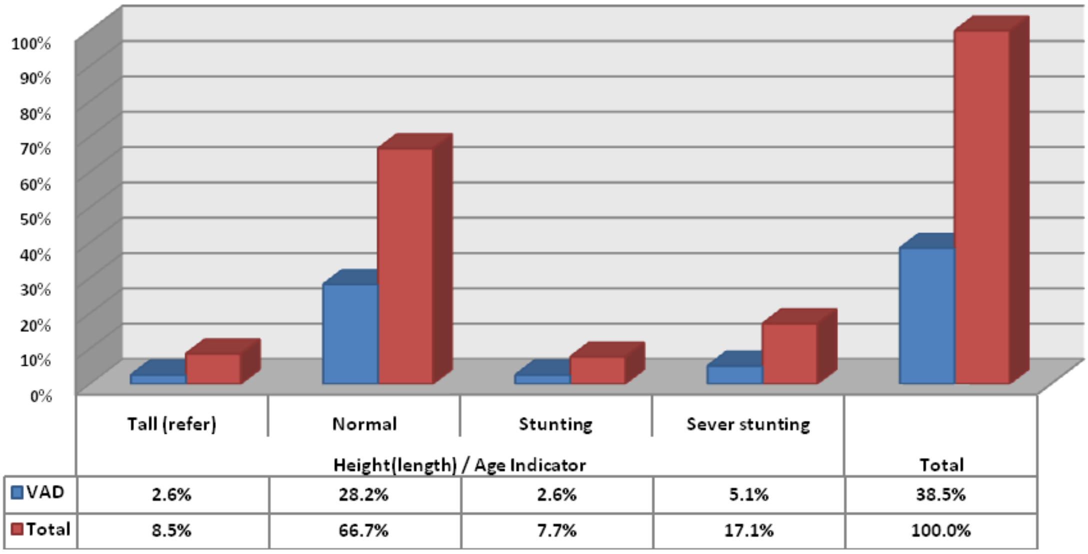
	Obese	overweight	Risk of overweight	Normal	wasting	sever wasting	Total
	Weight / Height(lengt) Indicator						Total
VAD	2.5%	.8%	1.7%	31.9%	.8%	.0%	37.8%
Total	5.9%	7.6%	9.2%	73.9%	2.5%	.8%	100.0%

Figure (15): Distribution of VAD cases among Weight/Height (length) indicator categories



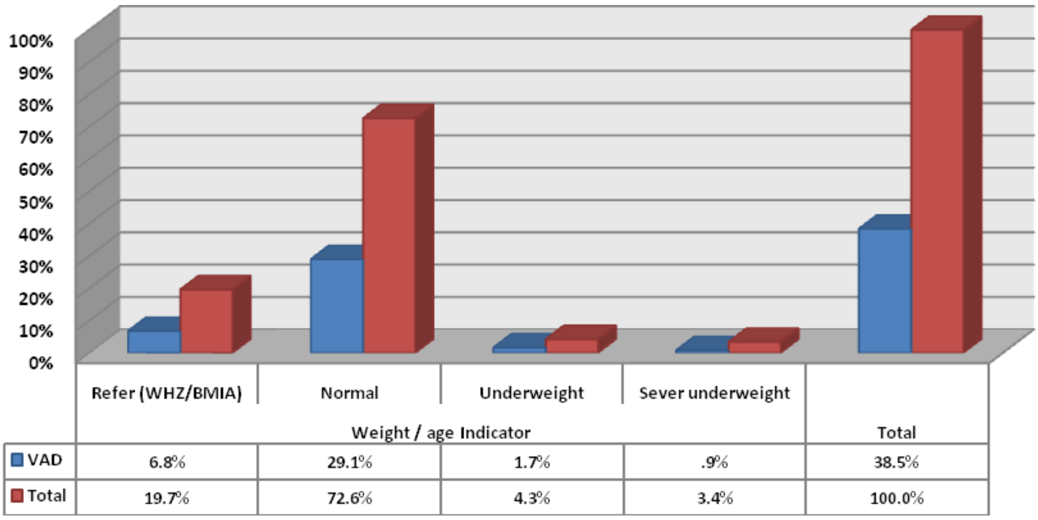
	Obese	overweight	Risk of overweight	Normal	wasting	sever wasting	Total
	BMI / Age Indicator						Total
VAD	3.4%	1.7%	.9%	31.9%	.9%	.0%	38.8%
Total	9.5%	6.9%	10.3%	70.7%	1.7%	.9%	100.0%

Figure (16): Distribution of VAD cases among BMI / Age indicator categories.



	Tall (refer)	Normal	Stunting	Sever stunting	Total
	Height(length) / Age Indicator				
VAD	2.6%	28.2%	2.6%	5.1%	38.5%
Total	8.5%	66.7%	7.7%	17.1%	100.0%

Figure (17): Distribution of VAD cases among Height (length) / Age indicator categories.



	Refer (WHZ/BMIA)	Normal	Underweight	Sever underweight	Total
	Weight / age Indicator				
VAD	6.8%	29.1%	1.7%	.9%	38.5%
Total	19.7%	72.6%	4.3%	3.4%	100.0%

Figure (18): Distribution of VAD cases among Weight / age Indicator categories.

Conclusions

This study shows that subjects in the 3 groups (preschool children, pregnant and lactating women) are at risk of VAD and anemia; nearly half of them had the co-occurrence of VAD and anemia. A close association between vitamin A deficiency and anemia with A correlation coefficient between SR and Hb concentrations was significant (N=490, r=0.533, P<0.0001) as proved in many studies.

Recommendations

1) Food-based approach to prevent and control of VAD including dietary diversification , nutrition education and fortification of staple and value-added foods through increasing the variety and frequency of micronutrient rich food sources and improved food preparation and cooking methods .
2) Supplementation with vitamin A capsules with increasing interest in a multimicornutrient supplement to prevention and treatment vitamin A deficiency.
3) Effective nutrition education and information on health and nutrition to increase the demand for consumption of such foods using the available mass and multimedia like television and radio and supporting behavioral change to improve micronutrient intake.
4) Public health interventions such as immunization, adding vitamin A supplementation to national immunization days, promotion of breast-feeding and treatment of infectious diseases.
5) Iron supplementation is the most common strategy currently used to control iron deficiency in Iraq. It should also be considered as a preventive public health measure to control iron deficiency in populations at high risk of iron deficiency anemia.
6) Establish a nation-wide survey on VAD and anemia and create a national database on micronutrient deficiencies.
7) Further investigation is needed to identify risk factors and evaluate interventions to address nutrition programs towards preventing and controlling VAD and anemia nation-wide.

References

1. World Health Organization.VtaminA supplements , a guide to their use in the treatment and prevention of vitamin A deficiency and xerophthalmi . Report of a WHO/UNICEF/ IVACG task force, second edition. Geneva: WHO, 1997

2. West KP Jr: Extent of vitamin A deficiency among preschool children and women of reproductive age. J Nutr 2002, 132:2857S-2866S

3. West KP Jr: Vitamin A deficiency disorders in children and women. Food Nutr Bull 2003, 24(4 Supple):S78-90.

4. Reifen R: Vitamin A as an anti-inflammatory agent. Proc Nutr Soc 2002, 61(3):397-400.

5. Maurin JF, Renard JP: Ocular manifestations of vitamin A deficiency and their prevention. 1997, 74:21-42.

6. Villamor E, Fawzi WW: Vitamin A supplementation: implications for morbidity and mortality in children Infect Dis 2000, 182:S122-133.

7. Bieri JG, Tolliver TJ, Catignani GL: Simultaneous determination of alpha-tocopherol and retinol in plasma or red cells by high pressure liquid chromatography. Am J Clinical Nutrition 1979, 32(10):2143-2149.

8. Michel j.Gibney, Barrie m.margetts, Jon m.kearney and Lenore Arab. Public health nutrition, The Nutrition Society, 2006.

9. Control of vitamin A deficiency and xerophthalmia; World Health Organ Tech Rep Ser 1982, 672:1-70.

10. Shamah T, Villalpando S: The role of enriched foods in infant and child nutrition. Br J Nutr 2006, 96:S73-77.

11. Huiming Y, Chaomin W, Meng M: Vitamin A for treating measles in children.

Cochrane Database Syst Rev 2005, (4):CD001479

12.Qiu X, Chen X, Yang S, Huang Y, Ou P, Chen Q, Zhang R: Epidemiological Study on Prevalence of Vitamin A Deficiency and Its Influential Factors in Children Under 5 years old in Fujian Province. ZhongGuo Er Tong Bao Jian Za Zhi 2005, 13(1):1-3.

13. Konawa YA, Muslimatun S, Achadi EL, Sastroamidjojo S. Anemia and iron deficiency anemia among young adolescent girls from the peri urban coastal area of Indonesia. Asia Pac J Clin Nutr 2006; 15:350-356

14. Allen LH. To what extent can food-based approaches improve micronutrient status? Asia Pac J Clin Nutr 2008; 17 Supple 1:103-105.

15. World health report 2002 — Reducing risks, promoting healthy life. Geneva: World Health Organization; 2002.

16. WHO/UNICEF/UNU, eds. Iron deficiency anemia assessment, prevention, and control: a guide for programme managers. Geneva, Switzerland: World Health Organization, 2001.

17.Hodges RE, Sauberlich HE, Canham JE, Wallace DL, Rucker RB, Mejia LA & Mohanram M (2000): Hematopoietic studies in vitamin A deficiency. Am. J. Clin. Nutr. 31, 876– 885.

18. Mejı´a LA, Hodges RE & Rucker RB (2003): Clinical signs of anemia in vitamin A-deficient rats. Am. J. Clin. Nutr. 32, 1439 – 1444. World Health Organization. 19.Indicators for assessing vitamin A deficiency and their application in monitoring and evaluating intervention programmes. Report of a WHO/UNICEF Consultation, Geneva, 9-11 November 1992. Review version. Geneva: WHO, 1994

20. Molla A, Khurshid M, Molla AM, Badruddin SH, Hendricks K &Snyder JD (2003b): Is anemia an accurate predictor of vitamin A status in Pakistani children? Am. J. Trop. Med. 49, 276 – 279.

21. Mohanram M, Kulkarni KA & Reddy V (2002): Hematological studies in vitamin A deficient children. Int. J. Vit. Nutr. Res. 47, 389 – 393.

22. Fazio-Tirrozzo G, Brabin L, Brabin B, Agbaje O, Harper G & Broadhead R (2001): A community based study of vitamin A and vitamin E status of adolescent girls living in the Shire valley, Southern Malawi. Eur. J. Clin. Nutr. 52, 637 – 642.