

Vitamin E Level In Friedreich's Ataxic Phenotype Patients In Four Major Hospitals In Baghdad

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ABSTRACT

Background: Friedreich ataxia (FRDA) is the most common form of inherited ataxia, comprising one-half of all hereditary ataxias with a carrier rate between 1 in 60 to 1 in 90 and with a disease prevalence of 1 per 29,000. It can occur in two forms the classic form or in association with a vitamin E dependent ataxia. The precise role of Vitamin E in the nervous system is unknown; An Oxidative attack is suspected to play a role in Ataxia with Vitamin E deficiency, as well as in Friedreich ataxia. Vitamin E is the major free-radical-trapping antioxidant.

Objective: The objectives of the study is to asses vitamin E level in patients with Friedreichs ataxia phenotype in Iraqi patients.

Type of the study: Cross-sectional study.

Method: This study was conducted at the neuroscience hospital and Baghdad teaching hospital during the period from the 1st of November 2013 through November 2014. Forty patients with friedreich's ataxia attended in neuroscience hospital and Baghdad teaching hospital during this period; there was 12 male, 20 female patients and their age range between (4-50) years.

Results: Regarding the level of vitamin E in patients in the present study, The study revealed that mean level of vitamin E for Friedreich ataxia patients was (10.92 µg/ml) ranging from (8-18 µg/ml), while in the control group the mean was (28.06µg/ml) ranging from (22-36µg/ml), the difference in mean level was found to be statistically significant ($p=0.0001$.) Thirty percents of (FRDA) patients was (8-9.9µg/ml), while half of patients (50%) were within (10-11.9 µg/ml), and low percentage (15 %) of patients were within (12-13.9 µg/ml). The rest (5%) were had vitamin E level equal or more than 14 µg/ml. these finding revealed that majority of Friedreich ataxia patients were found with low level of vitamin E (32 patients(80%)), and low percentage

with normal level (8 patients(20%)) Patients with Friedreich ataxia were found to have a comparable level $10.47\pm 1.79(8.4-13.5)$, $11.48\pm 2.61(9.4-18)$ and $10.66\pm 1.26(9-12.5)$ for those with less than 10 years, 10-19 years, equal or more than 20 years of age respectively ($p=0.382$), while it was found to have a significantly decreasing level with advance in the age for the control group, $30.57\pm 4.61(24.5-36)$, $27.46\pm 3.16(23.7-33.5)$, $25.50\pm 3.03(22.9-29)$ for those less than 10 years, 10-19 years, 20 years or more respectively ($p=0.006$.) Vitamin E level in relation to Gender showed that Friedreich ataxia male patients had significantly higher vitamin E level compared to females [$12.05\pm 2.66(9.0-18.0)$ compared to $10.17\pm 1.07(8.4-12.0)$] ($p=0.003$), while relation to gender in the control group was not of significant value statistically [$27.08\pm 2.90(22.9-30.0)$ for male compared to $29.04\pm 4.97(23.7-36)$ for females] , $p=0.136$.

Conclusion: In the light of the results of the present study, the following conclusions were made: The level of vitamin E in friedreichs ataxic phenotype patients was subnormal than normal range representing 80% of the sample selected for the test. The level of vitamin E in male patients of freidriechs ataxic phenotype patients was higher than female patients.

Keywords: Friedreich , Ataxia , Vitamin E.

*Al-Kindy College Medical Journal 2017: Vol. 13 No. 1
Page: 132-136*

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*Received 15th Feb 2017, accepted in final 30th March 2017
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Friedreich ataxia (FRDA) is the most common form of inherited ataxia, and is the most common form of the autosomal recessive ataxias comprising one-half of all hereditary ataxias¹. It can occur in two forms the classic form or in association with a vitamin E dependent ataxia. Clinical features include progressive limb and gait ataxia, dysarthria, loss of joint position and vibration senses, absent tendon reflexes in the legs, and extensor plantar responses². The onset of Friedreich ataxia is early; it typically presents in children aged (8-15 years) and almost always presents before age 20 year, Friedreich ataxia is a relatively common disorder. Overall, the Friedreich ataxia carrier rate has been estimated recently to be 1 in 60 to 1 in 90, with a disease prevalence of 1 per 29,000. Loss of ambulation typically occurs 15 years after disease onset. More than 95% of patients are wheelchair bound by age 45 years.

The average age of age of death was 37.7 years, the most common cause of death in Friedreich ataxia patients is the cardiac failure and arrhythmia. Classic Friedreich ataxia is the result of a gene mutation at the centromeric region of chromosome 9 (9q13-21.1) at the site of the gene encoding for the frataxin (210-amino-acid protein). In a normal chromosome, this trinucleotide sequence is repeated up to 50 times. In patients with FA, this sequence is repeated at least 200 times and often more than 1000 times, Variability in the clinical presentation of Friedreich ataxia may be explained by the extent of this trinucleotide expansion. Ataxia with Vitamin E deficiency (AVED), is inherited with an autosomal recessive pattern, The abnormal gene was mapped to chromosome 8q and identified as the gene encoding for α -tocopherol transfer protein (α -TTP) (3,4,5,6,7,8). It is difficult to distinguish on the basis of

clinical features between Ataxia with Vitamin E deficiency patients in whom vitamin E supplementation may be beneficial and those with classic Friedreich ataxia^{9,10}. The precise role of Vitamin E in the nervous system is unknown, Oxidative attack is suspected to play a role in Ataxia with Vitamin E deficiency, as well as in Friedreich ataxia. Vitamin E is the major free-radical-trapping antioxidant, the deficit of these factors may cause excess hydroxyl-radical generation, leading to molecular and tissue damage¹¹. Vitamin E deficiency may affect nervous tissue in other ways, including overproduction of cytolytic phospholipids and disturbance of brain monoamine metabolism^{12,13}. Aim of study is to assess vitamin E level in patients with Friedreich's ataxia phenotype in Iraqi patients.

Method :A cross sectional study with analytic elements was done for patients' cases who were conducted at the neuroscience hospital and Baghdad teaching hospital during the period from the 1st of November 2013 through November 2014. Forty patients with Friedreich's ataxia attended in neuroscience hospital and Baghdad teaching hospital during this period; there was 12 male, 20 female patients and their age range between (4-50) years.

Inclusion criteria: Any patients with ataxia and skeletal deformity and optic atrophy.

Exclusion criteria: any patients with abnormal MRI suggestive of Ischemia, Infection, Demyelination, Tumor.

Control group: consist of 40 healthy persons, age range between (8-25) years, (20 male-20 female) recruited from hospital staff and their relatives to test for the level of vitamin E. Examination of patients and control done by specialist neurologist in Baghdad teaching hospital and neuroscience hospital, each patient sent for vitamin E assessment at the same lab according to shape below (see session 2.3), all the patients had previous MRI and were revised by researcher. Serum concentration of vitamin E are measured using high performance liquid chromatography (HPLC) with photodiode array detection, small volume (100 ml) butyrate, the micronutrients are extracted from the aqueous phase into hexane and dried under vacuum. The extract is dissolved in ethanol and acetonitrile and is filtered to remove an insoluble material. An aliquot of the filtrate is injected onto a C18 reversed phase column and isocratically eluted with a mobile phase consisting of equal parts of ethanol and acetonitrile. Absorbance of these substances in solution is linearly proportional to concentration (within limits), thus spectrophotometric methods are used for quantitative analysis. Three wavelengths approximately corresponding to absorption maxima 300, 325, 450 nm, are simultaneously monitored. Achromatograms are recorded. Quantitation is accomplished by comparing the peak height or area of analyte in the unknown with peak height or area of unknown amount of same analyte in a calibrator solution. Calculations are corrected based on the peak height or area of the internal standard in the unknown compared with the peak height or area of internal standard in a calibrator solution. Retinol and retinyl butyrate at 325nm, tocopherol are compared with retinyl butyrate at 300 nm. A questionnaire form had been

developed and tailored to insure proper data collection and prevent any misunderstanding (appendix 1). All questionnaire forms were filled by the researcher by obtaining the data from the files of the patients in the neurological ward in the hospital. Data were analyzed using SPSS Statistics, (Statistical Packages for Social Sciences) version 20. Data presented in forms of numbers and percentages in tables as well as figures. Chi-square test was used to evaluate the association between compliance & each of the following variables: age, gender, age at diagnosis, education years, duration of treatment, type of seizure, EEG findings, family history & past medical history and between follow up & the same variables. A (P value ≤ 0.05) was considered statistically significant.

Result :Regarding the age of patients in the present study, the table 3.1 showed that (40 %) of patients were between (10-19) years age, (35%) of patients were less than (10) years, and (25%) represent those more than (20) years. Regarding the ratio between sexes, there was a little higher female ratio (60%) whereas (40%) were males.

Table (3-1) The distribution of patients according to age and gender

		FRDA		Control		P value
		No	%	No	%	
Age (years)	< 10	14	35.0	14	35.0	-
	10---19	16	40.0	16	40.0	
	= > 20	10	25.0	10	25.0	
Gender	Male	16	40.0	20	50.0	0.369
	Female	24	60.0	22	50.0	

Table (3-2) The mean and standard deviation of level of vitamin E in patients and control healthy group

Vitamin E level $\mu\text{g/mL}$	G r o u p s	
	FRDA	CONTROL
Number	40	40
Mean \pm SD	10.92 \pm 2.07	28.06 \pm 4.14
SEM	0.33	0.65
Range	(8.4-18.0)	(22.9-36.0)
Percentile 05 th	8.70	22.90
	9.60	24.50

	11.00	28.00
	11.10	30.00
	15.75	36.00
	18.00	36.00
P value	0 . 0 0 0 1 *	
*Significant difference between two independent means using Student-t-test at 0.05.		

Regarding the level of vitamin E in the patients in this study, it revealed that mean level of vitamin E for Friedreich ataxia patients was (10.92 µg/ml) ranging from (8-18 µg/ml) and in the control patient the mean was (28.06µg/ml) ranging from (22-36µg/ml), the difference in mean level was to be found statistically significant (p=0.0001) ;see the table (3-2).

Table (3-3)Level of vitamin E in (FRDA) patients and control healthy person.

	F R D A		C O N T R O L		P value
	No	%	No	%	

Vitamin E level (µmol/dl)	8 ---	12	30.0	-	-	-
	10 ---	20	50.0	-	-	
	12 ---	6	15.0	-	-	
	=>14	2	5.0	40	100	
Vitamin E level (µmol/dl)	Low	32	80.0	-	-	-
	Normal 12-64 µm/dl	8	20.0	40	100	
	High	-	-	-	-	
*Significant difference between proportions using Pearson Chi-square test at 0.05.						

This table(3-3) shows that vitamin e level in (30%) of (FRDA) patients was (8-9.9µg/ml), (50%) were with (10-11.9 µg/ml), (15 %) were of (12-13.9 µg/ml) , and (5%) were with vitamin E level of equal or more than 14 µg/ml.

These finding revealed that majority of Friedreich ataxia patients were found with low vitamin E (32 patients; 80%), and low percentage with normal level (8 patients; 20%).

Table (3-4)The level of vitamin E for the (FRDA) and control according to age and gender.

		V i t a m i n E l e v e l (m m o l / d l)				P v a l u e
		N o	F R D A	N o	C o n t r o l	
Age (years)	< 1 0	1 4	10.47±1.79 (8.4-13.5)	1 4	30.57±4.61 (24.5-36.0)	0.0001*
	1 0 --- 19	1 6	11.48±2.61 (9.4-18.0)	1 6	27.46±3.16 (23.7-33.5)	0.0001*
	= > 2 0	1 0	10.66±1.26 (9.0-12.5)	1 0	25.50±3.03 (22.9-29.0)	0.0001*
	P v a l u e		0 . 3 8 2		0 . 0 0 6 #	
Gender	M a l e	1 6	12.05±2.66 (9.0-18.0)	2 0	27.08±2.90 (22.9-30.0)	0.0001*
	F e m a l e	2 4	10.17±1.07 (8.4-12.0)	2 0	29.04±4.97 (23.7-36.0)	0.0001*
	P v a l u e		0 . 0 0 3 *		0 . 1 3 6	
D a t e w e r e p r e s e n t e d a s M e a n ± S D (R a n g e)						
#Significant difference between three independent means using ANOVA test at 0.05.						
*Significant difference between two independent means using Student-t- test at 0.05.						

Figure (3-1) the level of vitamin E for the (FRDA) and control according to age and gender.

The results in the table 3.4 showed that level of vitamin E were (10.47±1.79) ,(11.48±2.61) and (10.66±1.26) in patients less than 10 years , patients between 10-19 years, and for those equal or more than 20 years of age respectively, (p=0.382), See the table (3-4).The level of vitamin E in control patients were (30.57±4.61), (27.46±3.16) and(25.50±3.03) for patients less than 10 years age, patients between 10-19 years, and for those

equal or 20 years or more respectively and it was statically significant, (p=0.006),see the table (3-4).The level of vitamin E in friedreichs ataxic phenotypic patients according to the Gender showed that male patients had higher vitamin E level (12.05±2.66) compared to females(10.17±1.07) and it was statically significant(p=0.003),see the table (3-4).The level of vitamin E in relation to the gender in the control group

showed that female patients were (29.04±4.97), where as male patients were (22.9-30.0), the result was not statistically significant ($p=0.136$), see the table (3-4).

Discussion: Friedreich's ataxia is a relatively common disorder with significant morbidity associated with a range of deleterious consequences, including higher morbidity, restriction on social activities, and stress on the patient's family members and caregivers. It is also a great economic burden for the society through expenditures in healthcare and unemployment. The patients age range with Friedreich ataxia phenotype in this study who were of (10-19) years age comprise (40%) of total number of patients, where as those less than 10 years represent (35%), and those more than 20 years represent (25%), which means that most of patients ages was below 25 years, that goes with typical age presentation in Friedreich ataxia phenotype patients, and this study was similar to the results done by de michele *et al* & fortuna *et al* 14,15. Regarding the ratio between male and female in Friedreich ataxic phenotype patients, the percentage of female were (60%), whereas male were 40% in this study.

The present study showed that (FRDA) phenotype patients had a decreased level of vitamin E, representing (80%) of total patients, and this result was close to J. M. Copper *et al* who found ninety one percentage of their series had low level of vitamin E16. The low level in vitamin E may be due to increased oxidant stress observed in blood and urine from FRDA patients, and the decrease in frataxin protein lead to mitochondrial damage leading to increase oxidative damage which in turn increased the turnover of vitamin E. The level of vitamin E in different age groups was low, there was no statistical difference between different age groups, and that goes with the finding in the study done by J.M.Copper *et al* 17. Gender factor in this study showed (FRDA) male patients had significantly higher vitamin E level compared to females, this in contrast to the results seen in the control groups in the present study which showed no statistical difference.

Limitations of this study were that disease prevalence is relatively low and leads to difficulty to collect large number of patients. There was also difficulty in delivering sample of blood to the lab in appropriate time, that makes false positive result because of sensitivity to the hot environment, and the time to take the sample to the lab since the lab is away from the patients. It's a hospital-based study and its results may not reflect the real picture in the general population. Another limitation was that most of Friedreich's ataxic phenotype patient attend the private clinic and not attending the hospital that lead to low number of patients in this study.

Conclusion: In the light of the results of the present study, the following conclusions were made: 1-The level of vitamin E in Friedreich's ataxic phenotype patients was subnormal than normal range representing 80% of the sample selected for the test. 2-The level of vitamin E in male patients of Friedreich's ataxic phenotype patients was higher than female patients.

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