difference was 58%, which was not statistically significant.

To the best of our knowledge, the present study is the first to systematically investigate interindividual seasonal variations of CRP in several large populations. In two studies, seasonal variation of CRP was investigated on an intraindividual basis. Woodhouse et al. (2) reported higher CRP concentrations in winter with a peak in March, and Crawford et al. (13) found a significant seasonal variation of CRP with a peak in late February. The lack of seasonal variability of CRP in the present study may be somewhat contradictory to a previous study which may be somewhat contradictory to a previous report from our group (8) and to others (2, 13), in which seasonal variations of a variety of acute-phase proteins, such as fibrinogen, PAI-1, plasminogen, and α1-glycoprotein, have been observed. However, in contrast to the major acute-phase reactant CRP, these coagulation proteins are not exclusively related to the acute-phase response.

The predictive value of CRP for cardiovascular events has been consistently established in a variety of prospective studies (3, 4), and highly sensitive assays for CRP are now widely available with low analytical variability. In a recently published study (14), Meier-Ewert et al. demonstrated that baseline CRP concentrations are not subject to time-of-day variation. In the present study, we found no convincing evidence for seasonal variation of CRP; thus there should be no concern about misclassification of participants in population studies and in clinical practice measured during various seasons.

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Friedreich ataxia (FA) is an autosomal recessive spino-cerebellar syndrome with onset before age 25, characterized by progressive cerebellar ataxia, dysarthria, areflexia, sensory loss in lower limbs, pyramidal weakness, and Babinski signs (1). It is caused by an intronic expanded GAA repeat in the frataxin gene (2) located on chromosome 9q13-q21 (3). Investigating five Tunisian families with typical FA phenotype, Ben Hamida et al. (4) had excluded linkage to the locus of FA in two families and provided evidence for genetic heterogeneity of the disease. Patients belonging to families not linked to the locus of FA showed very low serum vitamin E (VE) with no evidence of lipid malabsorption.

Because it is difficult to distinguish on the basis of clinical features between AVED patients in whom VE supplementation may be beneficial (7, 13, 14) and those with classic FA, we assessed serum VE, total cholesterol (TC), and triglycerides (TGs) in our patients with FA clinical phenotype and their unaffected family members.

Serum Vitamin E and Lipid-adjusted Vitamin E Assessment in Friedreich Ataxia Phenotype Patients and Unaffected Family Members, Moncef Feki,1 Samir Belal,2 Habib Feki,3 Malek Souissi,1 Mahbouba Frih-Ayyed,4 Nazihya Kaabachi,1 Fayaşal Hentati,2 Mongi Ben Hamida,2 and Abderraouf Mebazaa1 1Laboratory of Biochemistry, Rabta Hospital, 1007 Tunis, Tunisia; 2Service of Neurology, National Institute of Neurology, 1007 Tunis, Tunisia; 3Service of Community Medicine and Epidemiology, Hedi Chaker Hospital, 3029 Sfax, Tunisia; 4Service of Neurology, Fattouma Bourguiba Hospital, 5000 Monastir, Tunisia; *address correspondence to this author at: Laboratoire de Biochimie Clinique, Hôpital La Rabta, 1007 Eljabbari, Tunis, Tunisia; fax 216-71-570-506, e-mail abderraouf.mebazaa@rns.tn.

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We studied 175 patients, belonging to 68 Tunisian families (age range, 4–52 years; 83 males, 86 females). All fulfilled the clinical criteria of the typical form of FA (1): age of onset before 20 and progressive cerebellar ataxia with dysarthria, areflexia, deep sensory loss in lower limbs, pyramidal weakness, and Babinski signs. None had hepatobiliary or pancreatic disease or any cause of fat malabsorption. Genetic linkage analysis, performed in all patients and relatives by polymorphic microsatellite repeats, showed linkage with FA locus markers (D9S202-D9S886-D9S888-D9S887) for 74 patients and with AVED locus markers (D8S510-D8S1228-D8S1178-D8S544-D8S553) for 101. We included 238 unaffected family members (107 parents and 131 siblings; age range, 5–80 years; 114 males, 124 females). Age-matched controls were selected from a group of 165 healthy volunteers not related to the patients (age range, 5–80 years; 78 males, 97 females). All study participants consumed a regular Tunisian diet, and none had knowingly received VE supplements within the last year.

Blood samples were collected into EDTA-containing tubes and plain tubes from fasting individuals and centrifuged without delay at 1500g for 10 min. Plasma was removed and stored at –80 °C, away from light, until VE analysis (within 1 year). Serum was kept at 4 °C for lipid assay (within 24 h).

VE was assessed by HPLC as described by Driskell et al. (15). Briefly, plasma was deproteinized in the presence of ethanol–butylated hydroxytoluene containing retinol acetate as an internal standard. VE was extracted with hexane and evaporated to dryness under a stream of nitrogen. The residues were redissolved in methanol–butylated hydroxytoluene and injected into a C18 reversed-phase column (Shimpack ODS-M). Mobile phase consisted of methanol (gradient grade; Merck) at a flow rate of 1.5 mL/min, and a VE peak was detected at 290 nm. The within-day (n = 20) and the long-term (n = 30) imprecision (CVs) were 4.2% and 4.5%, respectively, at a concentration of 23 μmol/L. TC and TGs were tested by enzymatic methods (Biomagreb). VE/TC and VE/TC+TGs ratios, which are better indicators of VE status (16), were calculated.

Serum VE was very low or undetectable in the 101 patients linked to the AVED locus. In the 74 others who were linked to the FA locus, VE was comparable to that of the controls. This permitted us to establish the diagnosis of AVED in the first group and FA in the second. Serum TC and TGs were in the reference intervals for all patients (Table 1). Biochemical liver tests, p-xylose test, and steatorrhea, performed on at least one patient per family, were within the reference interval.

Unaffected relatives of AVED patients showed a significant decrease of serum VE and lipid-adjusted VE compared with age-matched controls (3.61 ± 1.22 vs 4.57 ± 1.04 mmol/mol for VE/TC ratio; P <0.001). This mild decrease was also obvious in the parents of AVED patients, who are heterozygous for this autosomal recessive disease, in comparison with age-matched controls (Fig. 1).

In previous studies, serum VE was reported to be within the reference interval (4, 17, 18) or very low (4, 7–10) in patients with clinical features suggestive of FA. In our studies, serum VE was within the reference interval in patients linked to the FA locus and very low or undetectable in patients linked to the AVED locus. The normality of TC, TGs, and biochemical liver and p-xylose tests excludes secondary VE deficiency and confirms the diagnosis of AVED in our deficient patients. These data highlight the usefulness of serum VE assessment in FA phenotype. The genetic analysis is the gold standard for the diagnosis of FA, AVED, and other forms of ataxia, and the frataxin or α-TTP gene testing is superior to linkage studies. However, genetic testing is expensive and is often unavailable in developing countries for routine practice. Moreover, no conditions other than a-β-lipoproteinemia, chronic cholestasis, and large intestinal resection (which could be easily excluded) showed such very low VE concentrations similar to those observed in AVED.

AVED is caused by a mutation of the α-TTP gene (12), which makes the cytosolic hepatic α-TTP unable to incorporate α-tocopherol in lipoproteins and the liver incapable of secreting it in the plasma (19). The precise role of VE in the nervous system is unknown. Oxidative attack is suspected to play a role in AVED, as well as in FA. VE is the major free-radical-trapping antioxidant (20), and frataxin is a key element of the system controlling iron metabolism and free-radical generation in the mitochon-

| Table 1. Comparative values of serum VE, TC, and TGs in AVED and FA patients and age-matched controls. |
|-----------------|-----------------|-----------------|
|                 | AVED patients   | FA patients     | Age-matched controls |
|                 | (n = 101)       | (n = 74)        | (n = 90)              |
| Age, years      | 28.3 ± 10.2     | 24.2 ± 8.5      | 28.8 ± 9.9            |
| VE, μmol/L      | 0.64 ± 0.78a    | 19.49 ± 6.15    | 20.36 ± 5.93          |
| TC, μmol/L      | 4.30 ± 1.22     | 4.48 ± 1.12     | 4.54 ± 0.85           |
| TGs, μmol/L     | 1.23 ± 0.78     | 1.20 ± 0.68     | 1.00 ± 0.40           |
| a P <0.001 (vs controls). |              |                 |                      |

Fig. 1. Comparative distribution of VE, VE/TC, and VE/TC+TGs values in AVED parents (●; n = 63) and age-matched controls (□; n = 63).
dria (21). The deficit of these factors may cause excess hydroxyl-radical generation, leading to molecular and tissue damage. VE deficiency may affect nervous tissue in other ways, including overproduction of cytotoxic phospholipids (22) and disturbance of brain monoamine metabolism (23). Other roles of VE, such as modulation of necrosis xB and API transcription factors and protein kinase C (24), are probably essential for its neuroprotective effect.

Serum VE had rarely been investigated in unaffected family members of FA phenotype and was found to be within the reference interval in FA families (4) and decreased or within the reference interval in AVED families (4, 5, 25, 26). In our study, absolute and lipid-adjusted VE was lower in the parents of AVED patients, who are obligatory heterozygous. These lower concentrations are probably attributable to a mild decrease of hepatic α-TTP activity in heterozygotes.

In conclusion, AVED is relatively frequent in Tunisia and probably in North African countries. Measurement of serum VE would permit recognition of AVED patients, in whom serum VE supplementation may be beneficial. This could be particularly helpful in developing countries where genetic testing is not available and can be expensive. However, genetic testing is still the gold standard because VE could also be low in FA patients with malabsorption or nutritional problems. Serum VE, and especially lipid-adjusted VE, may be useful for screening of heterozygotes in AVED families.

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References


More Rapid Method for Simultaneous Measurement of Tryptophan and Kynurenine by HPLC, Andreas Laich,1 Gabriele Neurauter,1,2 Bernhard Widmer,1 and Dietmar Fuchs2,3

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The essential amino acid l-tryptophan is important in nitrogen balance and the maintenance of muscle mass and body weight in humans (1). Moreover, tryptophan is the precursor for the biosynthesis of the neurotransmitter serotonin (5-hydroxytryptamine). Insufficient availability of tryptophan may increase susceptibility for mental depression (2). On activation of cellular immunity, the T-cell-derived cytokine interferon-γ stimulates the enzyme indoleamine-(2,3)-dioxygenase (IDO) in various cells (3, 4). IDO catalyzes the initial step of tryptophan catabolism within the biosynthetic pathway of nicotin-