Measurement of total antioxidant status in biological fluids as indicator of the activity of the antioxidant system.

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INTRODUCTION

Oxidative damage has been implicated in the aetiology or pathogenesis of a large number of diseases, in tissue injury as well as in the ageing process.[1-3] The antioxidant system is a control mechanism in place to reduce free radical damage. The **antioxidant defenses** interact to form an **integrated system**[4] It is of great interest to measure the **total antioxidant status (TAS)** as an indicator of the functioning of the entire system as it is composed of a number of elements that exert their actions in different ways,[4,5] with different reaction kinetics and interactions.

The availability of TAS assay kits with the necessary standardisation of both procedures and reactants, enabling automation and comparison of the results from different studies, represents an useful tool for clinical and research applications. **A TAS assay kit for measuring total antioxidant capacity** under **standardized procedure and reactants** was developed. We report here two examples of its applicability to the quantitative determination of TAS in serum and plasma samples. One example refers to the TAS values comparison between geriatric and working population from the same geographical area, the other example examines the effect in TAS values of dietary vitamin supplementation.

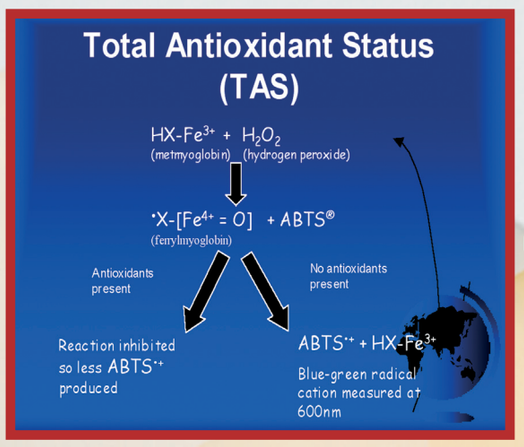
MATERIALS AND METHODS

Total Antioxidant Status (TAS) kit and Total Antioxidant Control were used (catalogue numbers NX 2332 and NX 2331 respectively, Randox Laboratories, Crumlin, UK). The assay was performed following manufacturer's specified assay conditions on a Cobas Fara II centrifugal analyzer (Roche, Switzerland).

Sample volume	**5 µl**
Wavelength	**600 nm**
Temperature	**37^{0}C**
Reading time	**3 min**
Unit	**mmol/l**
Linearity	**2.5 mmol/l**

• Assay principle outline:

Colorimetric method[6] based on the reaction shown below:



Total Antioxidant Status (TAS):

Interaction of ABTS (2,2'-azino-bis-[3-ethylbenzthiazoline sulphonate] with the **ferrylmyoglobin radical species** generated by the activation of metmyoglobin (XF-Fe^{III}) with H_2O_2.

↓

Formation of the **$ABTS^{\bullet+}$ radical cation**

Antioxidants in the added sample cause **suppression of $ABTS^{\bullet+}$** formation to a degree which is **proportional to their concentration**

• Statistics

Student's t-test (assuming equal variance) was used to determine whether differences between means were significant, with $p \leq 0.05$ taken as the significance level.

• Sample Population

1. **Geriatric and working populations from the same geographical area.**

Samples were collected as part of a routine health-screening programme (Vienna, Austria).

Geriatric population: serum samples were collected from 77 subjects. Mean age was 84.69 ±9.92 years (range: 61-102 years, 95% confidence limit=2.18 years). There was no significant sex difference in ages.

Working population: serum samples were collected from 156 individuals, mean age was 38.75 ±10.27 years (range: 19-59 years, 95% confidence limit = 1.61 years)

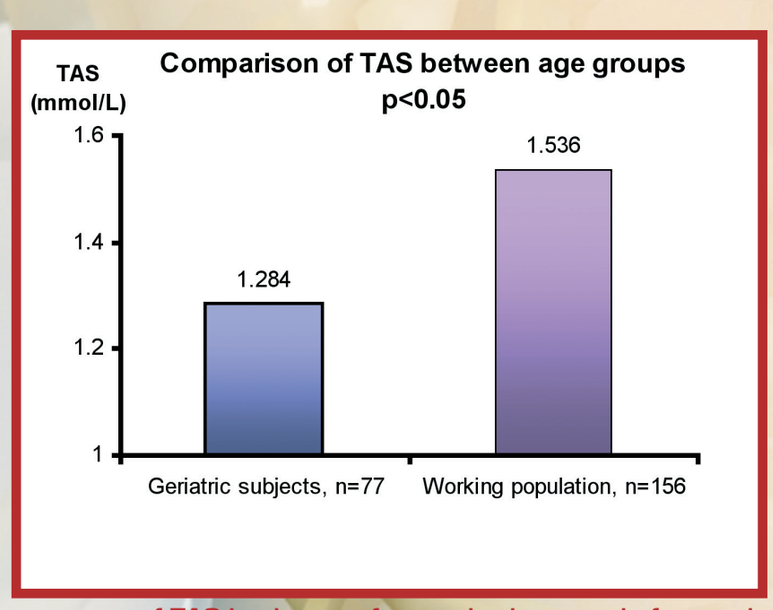
2. **Apparently normal volunteers before and after 60 days of vitamin supplementation.**

Plasma samples from each of **16 apparently normal volunteers** were assayed to determine a base line level prior to supplementation. Volunteers were then given one tablet containing antioxidant supplements each day for 60 days. At the end of this period, plasma samples were collected and assayed for determination of TAS levels post-supplementation.

Antioxidant supplementation contained: selenium (10 µg), vitamin A (450 µg), vitamin C (100 mg), vitamin E (91 mg), bioflavonoids (50mg).

RESULTS

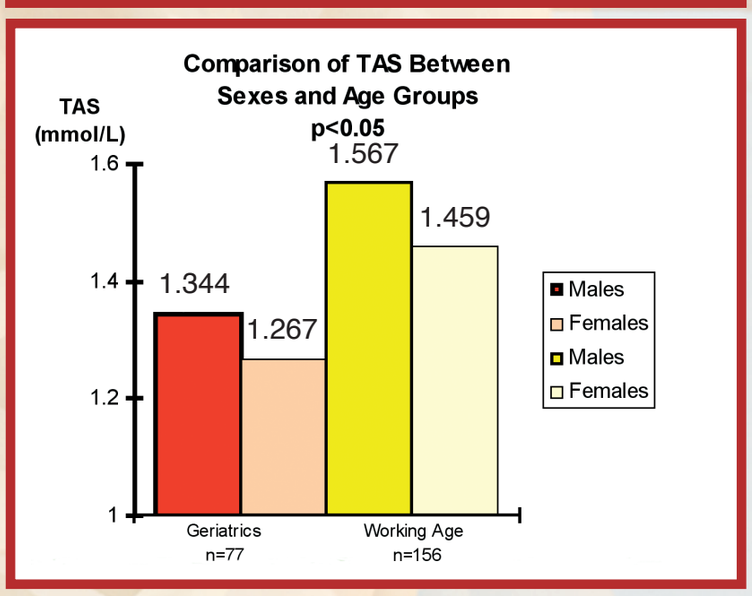
1. Measurement of TAS in geriatric subjects and comparison with working population from the same geographical area.



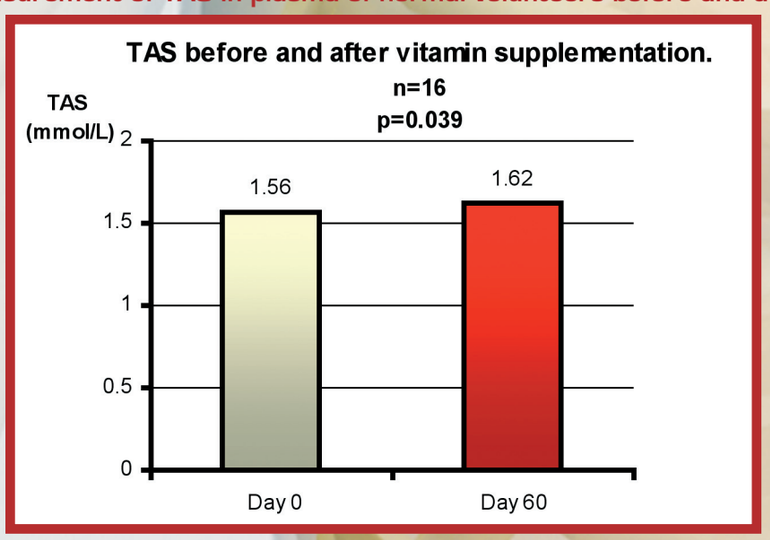
Mean TAS values in serum

Geriatric Subjects (n=77)
mean ± SD: 1.284 ± 0.15 mmol/L

Working population (n=156)
mean ± SD: 1.536 ± 0.11 mmol/L



2. Measurement of TAS in plasma of normal volunteers before and after 60 days of vitamin supplementation.



Mean TAS values in plasma

Before vitamin supplementation
mean ± SD: 1.56 ± 0.74 mmol/L

After 60 days post-supplemetation
mean ± SD: 1.62 ± 0.12 mmol/L

CONCLUSION

Data obtained by using this TAS assay kit, which offers standardization of both procedure and reactants, showed significant differences in the mean value of TAS between age and gender in a population from the same geographical area; as well as a significant increase in TAS after antioxidant supplementation in apparently normal volunteers.

When geriatric and working age groups from an Austrian population were compared in terms of mean TAS values, it was found that the geriatric population (range: 61-102 years) presented significant lower values than the working population and in both groups, the TAS values were significantly higher in male when compared with female subjects. This trend related to gender was also found in other studies carried out with different populations, whereas differences with age varied with the assayed populations.[7,8]

The measurement of TAS in plasma of normal volunteers before and after 60 days of antioxidant supplementation resulted in a significant increase of the mean TAS values post-supplementation. Using a different methodology, other authors reported an increase in plasma antioxidant capacity after consumption of controlled diets associated with antioxidant nutrients.[9]

In conclusion, this TAS assay kit represents a very applicable tool for the measurement of the overall antioxidant status in biological fluids. Though more in depth understanding would require longitudinal studies and correlations to other individual antioxidant parameters, TAS values obtained under standardized analytical conditions enable comparison of the results from different studies and can provide useful information for clinical and research applications.

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