TAS- TOS- OSI YÖNTEMLERİ

Venous blood was drawn into blood tubes and serum was separated from the cells by centrifugation at 1500 g for 10 min, and theserum samples were stored at –80 °C until the analyses.

Analytical methods

TOTAL ANTIOXDANT STATUS (TAS)

TAS levels were measured using commercially available kits (Relassay, Turkey). The novel automated method is based on the bleaching of characteristic color of a more stable ABTS (2,2 ’ - Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical cation by antioxidants. The assay has excellent precision values, which are lower than 3%. The results were expressed as mmol Trolox equivalent/L (Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radicalcation. Clin Biochem 2004;37:277-85.)

TOTAL OXIDANT STATUS (TOS)

TOS levels were measured using commercially available kits (Relassay, Turkey. In the new method, oxidants present in the sample oxidized the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction was enhanced by glycerol molecules abundantly present in the reaction medium. The ferric ion produced a colored complex with xylenol orange in an acidic medium. The color intensity, which could be measured spectrophotometrically, was related to the total amount of oxidant molecules present in the sample. The assay was calibrated with hydrogen peroxide and the results were expressed in terms of

micromolar hydrogen peroxide equivalent per liter (µmol H2O2 equivalent/L). (Erel O. A new automated colorimetric method for measuringtotal oxidant status. Clin Biochem 2005;38:1103-11.).

OXIDATIVE STRESS INDEX (OSI)

The ratio of TOS to TAS was accepted as the oxidative stress index (OSI). For calculation, the resulting unit of TAS was converted to µmol/L, and the OSI value was calculated according to the following Formula : OSI (arbitrary unit) =

TOS (µmol H2O2 equivalent/L) / TAC (µmol Trolox equivalent/L). (1-3).

1. Yumru M, Savas HA, Kalenderoglu A, Bulut M, Celik H, Erel O. Oxidative imbalance in bipolar disorder subtypes: a comparative study. Prog Neuropsychopharmacol Biol Psychiatry. 2009 Aug 31;33(6):1070-4.

2. Kosecik M, Erel O, Sevinc E, Selek S. Increased oxidative stress in children exposed to

passive smoking. Int J Cardiol 2005;100:61–4.

3. (Harma M, Harma M, Erel O (2003) Increased oxidative stress in patients with hydatidiform mole. Swiss Med Wkly 133:563-536).

AST

Summary:

Aspartate aminotransferase (glutamate-oxaloacetate-transaminase) belongs to the transaminases, which catalyze the interconversion of amino acids and a-keto acids by transfer of amino groups. Aspartate aminotransferase is commonly found in human tissue. Although heart muscle is found to have the most activity of the enzyme, significant activity has also been seen in the brain, liver, gastric mucosa, adipose tissue, skeletal muscle, and kidneys. AST is present in both the cytoplasm and mitochondria of cells. In cases involving mild tissue injury, the predominant form of AST is that from the cytoplasm, with a smaller amount coming from the mitochondria. Severe tissue damage results in more of the mitochondrial enzyme being released. Elevated levels of the transaminases can signal myocardial infarction, hepatic disease, muscular dystrophy, and organ damage. The International Federation of Clinical Chemistry (IFCC) recommended in 1977 and 1980 standardized procedures for AST determination, including optimization of substrate concentrations, employment of TRIS* buffers, preincubation of combined buffer and serum to allow side reactions with NADH to occur, substrate start, and optional pyridoxal phosphate activation. In 2002 the IFCC confirmed their recommendation and extend it to 37°C. This method is derived from the IFCC reference method. *TRIS = Tris(hydroxymethyl)-aminomethane

Test principle:

UV test according to a standarrized method

Sample and addition of R1 (buffer)

Addition of R2 and start of reaction:

$$\alpha\text{-ketoglutarate} + \text{L-aspartate} \xrightarrow{\text{AST}} \text{L- glutamate} + \text{oxaloasetate}$$

AST is the enzyme which catalyzes this equilibrium reaction. The oxaloacetate in- crease is measured in a subsequent indicator reaction which is catalyzed by malate dehydrogenase.

$$\text{oxalacetate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{MDH}} \text{L-Malate} + \text{NAD}^+$$

In the second reaction, NADH is oxidized to NAD. The rate of decrease in NADH (Measured photometrically) is directly proportional to the rate of formation of oxaloasetate, and thus the AST activity.

ALT

Summary:

Alanine aminotransferase (glutamate-pyruvate-transaminase) belongs to the group of transaminases which catalyze the conversion of amino acids to the corresponding a-keto acids via the transfer of amino groups; they also catalyze the reverse process. Although higher activities exist in the liver, minor activity can also be detected in the kidneys, heart, skeletal muscle, pancreas, spleen, and lungs. Elevated levels of transaminases are indicative of myocardial infarction, hepatopathies, muscular dystrophy, and damage to internal organs. Increased ALT activity in the serum, however, is a rather specific indicator of damage to the liver parenchyma, while AST is not necessarily a liver-specific parameter. The International Federation of Clinical Chemistry (IFCC) recommended standardized methods for the determination of ALT with optimized substrate concentrations, use of TRIS buffer, simultaneous preincubation of serum with buffer (to avoid competing reactions with NADH), substrate start, and pyridoxal phos-phate activation.

The method described here is derived from the IFCC reference method.

Test principle:

UV test according to the IFCC method.

$$\text{L-Alanin} + \text{2-Oxoglutarate} \xleftrightarrow{\text{ALT}} \text{L-Glutamate} + \text{Pyruvat}$$

$$\text{Pyruvat} + \text{NADH} + \text{H}^{+} \xleftrightarrow{\text{LDH}} \text{D-Lactate} + \text{NAD}^{+}$$

The enzyme alanine aminotransferase (EC 2.6.1.2; L-Alanine:2-Oxoglutarate Aminotransferase, ALT or A1aAT; Glutamate Pyruvate Transaminase, GPT) catalyzes the tran- saminase reaction between L-Alanine and 2-Oxoglutarate. The pyruvate formed, is reduced to lactate in the presence of LDH. As the reactions proceed, NADH is oxidized to NAD^{+}. The disappearance of NADH per unit time is followed by measuring the decrease in absorbance at 340 nm.