YÖNTEMLER

TAS- TOS- OSI

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).

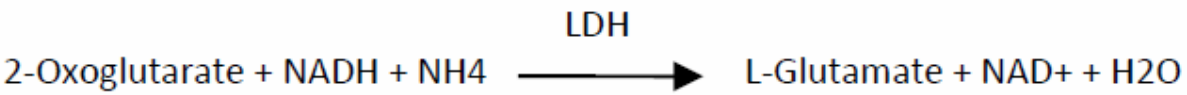
Thiol/disulfide homeostasis

tests were measured using a novel automatic and spectrophotometric method developed by Erel and Neselioglu* which is avaliable commercially (Rel Assay Diagnostics, Turkey) In this method, dynamic and reducible disulfide bonds in the samples were reduced to free functional thiol groups by using sodium borohydride. In order to prevent the reduction of unused reduced sodium borohydride to dithionite-2 nitrobenzoic (DTNB), NaBH4 was removed with formaldehyde. Native thiol (NT) and total thiol (TT) levels were determined after reaction with DTNB and their levels were measured ultimately. Half of the difference of the result obtained by the subtraction of native thiol amount from total thiol content indicated the disulfide (DS) level.

* Erel O, Neselioglu S. A novel and automated assay for thiol/disulphide homeostasis. Clin Biochem. 2014;47(18):326e332.

LDH

Optimised UV-Test according to DGKC LDH



Reagents – contents and concentrations:

R1:	
Triethanolamine pH 8.0	75 mmol/l
a-Ketoglutarate	10 mmol/l
Ammoniumacetate	150 mmol/l
EDTA	3.75 mmol/l
ADP	1.5 mmol/l
LDH	> 2.3 kU/l
R2:	
NADH	1.3 mmol/l

CK-MB

Test principle:

Immunological UV assay

- Sample and addition of R1 (buffer/enzymes/coenzyme/antibody)
- Addition of R2 (buffer/substrate) and start of reaction.

Human CK-MB is composed of two subunits, CK-M and CK-B which both have an active site. With the aid of a polyclonal antibody to CK-M, the catalytic activity of CKM subunits in the sample is inhibited to 99.6% without affecting the CK-B subunits.The remaining CK-B activity, corresponding to half the CK-MB activity, is determined by the CK-MB method analogous to total CK. As the CK-BB isoenzyme only rarely appears in serum and the catalytic activity of the CK-M and CK-B subunits hardly differ, the catalytic activity of the CK-MB isoenzyme can be calculated from the measured CK-B activity by multiplying the result by

CK

UV Test

- Sample and addition of R1
- Addition of R2 and start of reaction:

$$\text{Creatine phosphate} + \text{ADP} \xrightarrow{\text{CK}} \text{Creatine} + \text{ATP}$$

$$\text{ATP} + \text{glucose} \xrightarrow{\text{HK}} \text{Glucose-6-phosphate} + \text{ADP}$$

$$\text{Glucose-6-P} + \text{NADP} \xrightarrow{\text{G6PDH}} \text{Gluconate-6-P} + \text{NADPH+H}^{+}$$

Equimolar quantities of NADPH and creatine are formed at the same rate. The photometrically measured rate of formation of NADPH is proportional to the CK activity.

Troponin-I

This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to rat troponin-I. Standards or samples are added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for rat troponin-I and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain rat troponin-I, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the

concentration of Rat cTnT/TNNT2. You can calculate the concentration of Rat cTnT/TNNT2 in the samples by comparing the OD of the samples to the standard curve.