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).

Measurement of paraoxonase activity;

Paraoxonase activity was measured using

commercially available kits (Relassay, Turkey).

The rate of paraoxon hydrolysis (diethylpnitrophenylphosphate)

was measured by monitoring the increase of

absorption at 412 nm at 37 °C. The amount of generated p-nitrophenol

was calculated from the molar absorption coefficient at pH 8.5, which

was 18.290 M−1 cm−1 Paraoxonase activity was expressed as U/L

serum

**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.

Trigliserid

Triglycerides in the sample originates, by means of the coupled reactions described below, acoloured complex that can be measured by spectrophotometry.

Triglycerides + H2O lipase Glycerol + Fatty acids

Glycerol + ATP glycerol kinase Glycerol – 3 – P + ADP

Glycerol – 3 –P + O2 G-3-P-oxidase Dihidroxyacetone – P +H2O2

2 H2O2 + 4 – Aminoantipyrine + 4 – Chlorophenol G-3-P-oxidas Quinoneimine + 4 H2O

Cholesterol

Cholesterol is converted by oxygen with the aid of cholesterol oxidase to A4- Cholestenone and hydrogen peroxide. Hydrogen peroxide created forms a red dyestuff by reacting with 4-aminoantipyrine and phenol under the catalytic action of peroxidase. The color intensity is directly proportional to the concentration of cholesterol and can be determined photometrically.