**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).

**Paraoxonase- (PON-1)**

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.

**Cat:**

This colorimetric assay involves two steps. Sample is first incubated with a known amount of

hydrogen peroxide. Sample converts hydrogen peroxide to water and oxygen. The ratio is

proportional to the concentration of catalase. The enzyme is stopped and the remaining

hydrogen peroxide, following a fixed incubation period, is determined using a chromogen.

The resulting absorbance is measured at 405 nm and the obtained results are expressed as U/L.

**MDA**

The sample MDA level was determined by a method based

on the reaction with thiobarbituric acid (TBA) at 90–100\_C

[29]. In the TBA test reaction, MDA or MDA-like

substances and TBA react with the production of a pink

pigment with a maximum absorption at 532 nm. The

reaction was performed at pH 2–3 at 90\_C for 15 min. The

sample was mixed with two volumes of cold 10% (w/v)

trichloroacetic acid for the precipitation of protein. The

precipitate was pelleted by centrifugation, and an aliquot of

the supernatant was reacted with an equal volume of 0.67%

(w/v) TBA in a boiling water bath for 10 min. After

cooling, the absorbance was read at 532 nm. The results

were expressed as (µmol/ml).

**SOD**

The role of superoxide dismutase (SOD) is to accelerate the

dismutation of the toxic superoxide radical (02

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during oxidative energy processes, to hydrogen peroxide

and molecular oxygen.

This method employs xanthine and xanthine oxidase (XOD)

to generate superoxide radicals which react with

2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium

chloride (I.N.T.) to form a red formazan dye. The

superoxide dismutase activity is then measured by the

degree of inhibition of this reaction. One unit of SOD is that

which causes a 50% inhibition of the rate of reduction of

INT under the conditions of the assay.

**Ig-E**

Anti-human IgE antibodies when mixed with samples containing IgE, form insoluble complexes. These complexes cause an absorbance change, dependent upon the IgE concentration of the patient sample, that can be quantified by comparison from a calibrator of know IgE concentration.

SIGNIFICANCE

Based on antigen antibody reaction. IgE is an immunoglobulin with a molecular weight of approximately 190,000Da and is normally present in the blood in trace amounts. IgE antibodies are the chief immunoglobulin responsible for immediate hypersensitivity reactions in humans. Quantitative determination of IgE is maybe done by an immunoturbidimetric method, by automatic analysers or in manual. Mixing a sample with a precise Antigen to a solution having the corresponding anti-serum (Antibody), in a welldefined ratio, it is possible to have turbidity. Using our multipoint Calibrator, it is possible to prepare a Calibration Curve to refer, generally not rectilinear and not crossing the origin. Plotting on the Calibration Curve absorbance values and concentration for each single sample, may be determined the concentration of each sample

**Ig-A**

Immunoglobulins A (IgA) selectively react with an anti-IgA antibody and form an immunocomplex. The produced turbidity is proportional to the concentration of IgA in the sample, and can be measured at the wavelenght of 600 nm

**Ig-G**

Immunoglobulins G (IgG) selectively react with an antiIgG antibody and form an immunocomplex. The produced turbidity is proportional to the concentration of IgG in the sample, and can be measured at the wavelenght of 600 nm

**Ig-M**

Immunoglobulins M (IgM) selectively react with an antiIgM antibody and form an immunocomplex. The produced turbidity is proportional to the concentration of IgM in the sample, and can be measured at the wavelenght of 340 nm.