**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).

**MDA**

The tissue 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 nmol/g wet tissue.

(Relassay, Turkey)

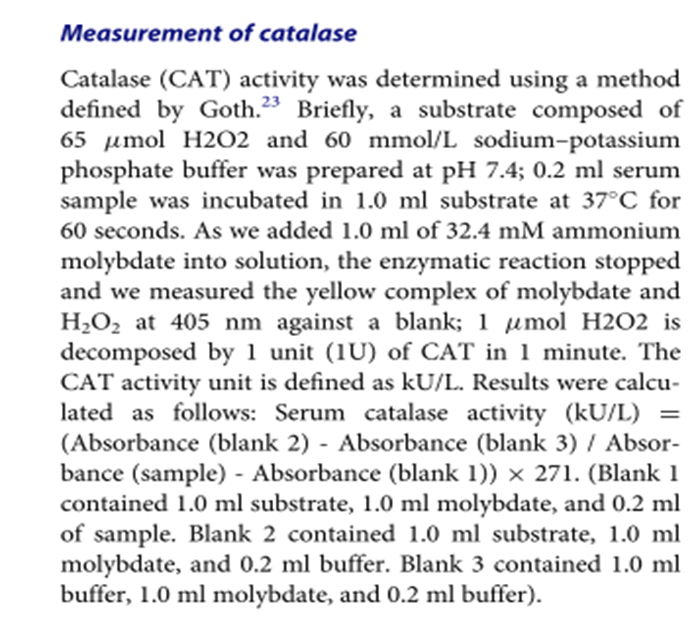
**NO Ölçümü**

NO ölçümü Griess yöntemi esas alınarak gerçekleştirilmiştir. NO çok kısa yarı ömürlü olduğu için hızla metabolitleri olan nitrit ve nitrata dönüşür. Nitrit doğrudan, nitrat ise nitrite indirgenerek Griess reaktifiyle ölçülür. Griess yönteminde; sülfanilamidin amino grubu asit ortamda, nitrit ile reaksiyona girerek diazotizasyona uğrar ve Naftiletilendiamin (NED) ile mor renkli bir azo ürünü oluşturur. Oluşan renk spektrometre cihazında 540 nm dalga boyunda okunduktan sonra nitrit standartları kullanılarak hazırlanmış kalibrasyon eğrisine göre plazma NO düzeyleri indirekt olarak hesaplanır.

**SOD**

The role of speroxide dismutase is to accelerate the dismutation of the toxic radical, produced during oxidative energy processes to hydrogen peroxide and molecular oxygen. This method employs xanthine and xanthine oxidase to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazan dye.. the superoxide dismutase activity is then measured by the degree of inhibiton of this reaction

**Catalase**



**GSH**

This kit uses enzyme-linked immune sorbent assay (ELISA) based on the Biotin double antibody sandwich technology to assay the Rat glutathione (GSH ). Add glutathione(GSH )to the wells, which are pre-coated with glutathione(GSH )monoclonal antibody and then incubate. After that, add anti GSH antibodies labeled with biotin to unite with streptavidin-HRP, which forms immune complex. Remove unbound enzymes after incubation and washing. Add substrate A and B. Then the solution will turn blue and change into yellow with the effect of acid. The shades of solution and the concentration of Rat glutathione (GSH ) are positively correlated.