• Tüm testler “RELASSAY” markadır. (Menşai: Türkiye)

• Testlerin çalışılacağı cihaz: Mindray marka BS300 model tam otomatik biyokimya cihazı

• Test metotları: Kolorimetrik

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-1 (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.

**MDA**

The Serum 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.

**Elisa Testleri: (Elabscience marka testler kullanıldı)**

**FSH**

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 FSH. 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 FSH 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 FSH, 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 FSH. You can calculate the concentration of Rat FSH in the samples by comparing the OD of the samples to the standard curve.

**LH**

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 LH. 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 LH 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 LH, 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 LH. You can calculate the concentration of Rat LH in the samples by comparing the OD of the samples to the standard curve.

**AMH**

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 AMH. 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 AMH 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 AMH, 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 AMH. You can calculate the concentration of Rat AMH in the samples by comparing the OD of the samples to the standard curve.

**8-OHdG**

This ELISA kit uses the Competitive-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with 8-OHdG. During the reaction, 8-OHdG in the sample or standard competes with a fixed amount of 8-OHdG on the solid phase supporter for sites on the Biotinylated Detection Ab specific to 8-OHdG. Excess conjugate and unbound sample or standard are washed from the plate, and Avidin conjugated to Horseradish Peroxidase (HRP) are added to each microplate well and incubated. Then a TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of stop solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of 8-OHdG in the samples is then determined by comparing the OD of the samples to the standard curve.

**Estradiol**

This ELISA kit uses the Competitive-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with E2. During the reaction, E2 in the sample or standard competes with a fixed amount of E2 on the solid phase supporter for sites on the Biotinylated Detection Ab specific to E2. Excess conjugate and unbound sample or standard are washed from the plate, and Avidin conjugated to Horseradish Peroxidase (HRP) are added to each microplate well and incubated. Then a TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of stop solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of E2 in the samples is then determined by comparing the OD of the samples to the standard curve.