**Yöntemler**

**TOTAL ANTIOXDANT STATUS (TAS) (mmol/L)**

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) (µmol/L)**

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).

**Malondialdehyde (MDA) nmol/L**

The tissue MDA level was determined by a method based

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

. 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)

**AST U/L**

UV test according to a standarrized method

Sample and addition of R1 (buffer)

Addition of R2 and start of reaction: AST

α-ketoglutarate + L-aspartate L- glutamate + oxaloasetate

AST is the enzyme which catalyzes this equilibrium reaction. The oxaloacetate in- crease is measured in a subsequent indicator reaction which is catalyzed by malate dehydrogenase.

MDH

oxalacetate + NADH + H+ L-Malate + NAD+

In the second reaction, NADH is oxidized to NAD. The rate of decrease in NADH

(Measured photometrically) is directly proportional to the rate of formation of

oxaloasetate, and thus the AST activity.

(AZD BioTec)

**ALT U/L**

UV test according to the IFCC method.

(AZD BioTec)

**GGT (Gamma GT) U/l**

Enzymatic colorimetric assay

• Sample and addition of R1 (Buffer/Glycylglycine)

• Addition of R2 (substrate) and start of reaction Gamma-glutamyltransferase transfers the g-glutamyl group of L-g-glutamyl-3-

carboxy-4-nitroanilide to glycylglycine. The amount of 5-amino-2-nitrobenzo-nate liberated is proportional to the GGT activity and can be determined photo-metrically.

(AZD BioTec)

**LDH**

Pyruvat + NADH + H+ ⎯⎯ D-Lactate+ NAD+

The enzyme alanine aminotransferase (EC 2.6.1.2; L-Alanine:2-Oxoglutarate Aminotransferase,

ALT or A1aAT; Glutamate Pyruvate Transaminase, GPT) catalyzes the tran- saminase reaction between L-Alanine and 2-Oxoglutarate.

The pyruvate formed, is reduced to lactate in the presence of LDH. As the reactions proceed,

NADH is oxidized to NAD+. The disappearance of NADH per unit time is followed by measuring the decrease in absorbance at 340 nm.

(AZD BioTec)

**Total Protein g/dl**

Colorimetric assay, Sample and addition of Reagent start of the reaction:

Divalent copper reacts in alkaline solution with protein peptide bonds to form the characteristic purple-colored biuret complex.

Sodium potassium tartrate prevents the precipitation of copper hydroxide and potassium iodide prevents auto reduction of copper. alkaline protein + Cu2+ solution Cu-protein complex

The color intensity is directly proportional to the protein concentration which can be determined photometrically.

(AZD BioTec)

**Albumin g/dl**

Colorimetric assay, endpoint method

• Sample and addition of R1

• Start of the reaction:

At a pH value of 4.1 albumin displays a sufficiently cationic character to be able to bind with bromocresol green (BCG), any anionic dyestuff, to form a blue-green complex.

pH 4.1 albumin + BCG albumin BCG- complex

The color intensity of the blue-green color is directly proportional to the albumin concentration and can be determined photometrical

(AZD BioTec)

**Bilirubin Direct mg/dl**

Jendrassik-Gróf method

In the presence of caffeine accelerator, total bilirubin couples with sulfanilic acid to form a red azobilirubin dye,

the color intensity which is proportional to the bilirubin concentration. Determination of direct bilirubin is performed without caffeine additive.

The addition of alkaline tartrate causes a transformation from the red azobilirubin dye to a blue dye and the absorbance maximum from 546nm to 578nm.

HCl

Sulfanilic acid+NaNO2 diazotized Sulfanic acid

HCl

Bilirubin + diazotized Sulfanic acid azobilirubin

(AZD BioTec)

**Bilirubin Total mg/dl**

Jendrassik-Gróf method

In the presence of caffeine accelerator, total bilirubin couples with sulfanilic acid to form a red azobilirubin dye,

the color intensity which is proportional to the bilirubin concentration. Determination of direct bilirubin is performed without caffeine additive.

The addition of alkaline tartrate causes a transformation from the red azobilirubin dye to a blue dye and the absorbance maximum from 546nm to 578nm.

HCl

Sulfanilic acid+NaNO2 diazotized Sulfanic acid

HCl

Bilirubin + diazotized Sulfanic acid azobilirubin

(AZD BioTec)

**Alkaline Phosphatase (ALP) U/L**

Colorimetric assay in accordance with a standardized method.

ALP, Mg2

p - Nitrophenylphosphate+ H2O Phosphate + p - Nitrophenol

In the presence of magnesium and zinc ions, p-nitrophenyl phosphate is hydrolyzed by phosphatases to form phosphate and p-nitrophenol.

In this process AMP serves as transient phosphate acceptor. The release of coloured p-nitrophenol is proportional to the ALP activity and can be measured photometrically.

(AZD BioTec)