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 H_2O_2 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 H_2O_2 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.

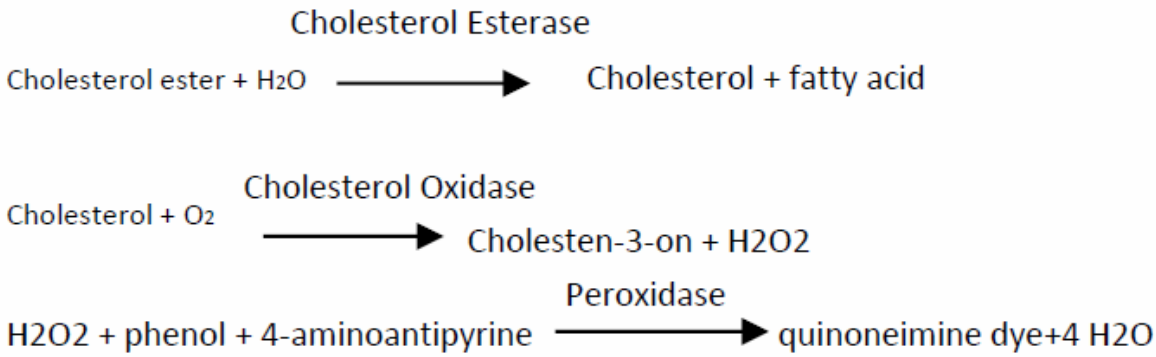
HDL:

Test principle:

Enzymatic colorimetric test

- Sample and addition of R1
- Addition of R2 and start of reaction

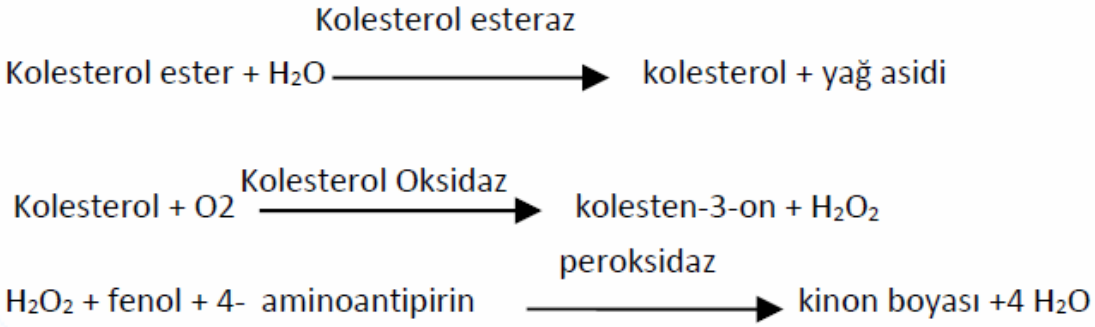
In the first step LDL, VLDL and Chylomicrons are eliminated and transformed to non reactive compounds and specific condition for the reaction. By the second reagent only the HDL-Cholesterol is subject to color reaction



LDL:

Test süreci:

İlk adımda, HDL, VLDL ve Şilomikronlar elimine edilir ve reaksiyon için özel koşulda reaktif olmayan bileşiklere dönüştürülür. İkinci reaktif sadece LDL-kolesterol renk reaksiyonudur.



Trigliserit:

Test principle:

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

$$\text{Triglycerides} + H_2O \xrightarrow{\text{lipase}} \text{Glycerol} + \text{Fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{glycerol kinase}} \text{Glycerol} - 3 - P + \text{ADP}$$

$$\text{Glycerol} - 3 - P + O_2 \xrightarrow{\text{G-3-P-oxidase}} \text{Dihidroxyacetone} - P + H_2O_2$$

$$2\,H_2O_2 + 4 - \text{Aminoantipyrine} + 4 - \text{Chlorophenol} \xrightarrow{\text{G-3-P-oxidas}} \text{Quinoneimine} + 4\,H_2O$$

Kolesterol:

Test principle:

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol} + \text{fatty acids}$$

Cholesterol esters are ceaved by the action of choesterol esterase to yield free choesterol and fatty acids

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{Cholesten-3-on} + H_2O_2$$

$$2H_2O_2 + \text{Phenol} + \text{4-Aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine dye} + 4\,H_2O$$

Cholesterol is converted by oxygen with the aid of cholesterol oxidase to A^4-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.