**Measurement of lipid peroxidase productions (Thiobarbituric acid reactive substances TBARS)**

***Ruiz Larrea et all (1994)***

The extent of lipid oxidation could be determined by measuring the levels of lipid peroxidation products as thiobarbituric acid reactive substances (TBARS), mainly malondialdehyde (MDA). One of the most commonly applied assays is the TBA test of Ruiz-Larrea et al (1994). According to this method, samples were heated with TBA at low pH and a pink chromogen was measured by its absorbance at 532 nm.

**Materiel:**

60 tubes x 2

**Reagents:**

- Reagent A: saturated TBA in 10% perchloric acid.

- Reagent B: 20% trichloroacetic acid (TCA)

**Working reagent:**

1 volume of reagent A was mixed with 3 volumes of reagent B.

**Protocol:**

4.5 ml of the **working reagent** was added to 0.5ml of the **supernatant.**

The mixture was incubated for 20 minutes in a boiling water bath, left to cool at room temperature and then centrifuged at 3000 rpm for 5 minutes.

The developed pink colour was measured at 532 nm.

A blank solution was set up at the same time using 4.5 ml of the working reagent and 0.5 ml distilled water and treated similarly as the samples.

Lipid peroxidation was expressed as µ moles MDA/ ml plasma using the extinction coefficient of MDA (ε = 1.53 105) at 532nm.

**Measurement of glutathione (GSH)**

***Beutler et al (1963)***

A simple and accurate method for the determination of **GSH** was used as described by Beutler et al (1963). This method is based upon the development of a relatively stable yellow color when 5.5’ dithiobis-2- nitrobenzoic acid (DTNB) is added to sulfhydryl compounds.

**Reagents:**

- Precipitating solution:

1.67g glacial metaphosphoric acid, 0.29g disodium ethylenediamine tetra acetic acid (EDTA) and 30 g sodium chloride were dissolved in 100 ml distilled water. This solution is stable for approximately 3 weeks at 40°C.

- Phosphate solution (0.3 mol):

Anhydrous Na2 HPO4 (42.6g) was dissolved in 1 litre bidsitlled water.

- DTNB reagent:

40mg DTNB were added to 100 ml 1% sodium citrate.

**Procedure:**

0.5ml sample, 2 ml phosphate solution and 0.25 ml DTNB reagent were pipetted into the cuvette. For each series of samples, a blank was prepared containing 0.5ml distilled water instead of the sample.

**Standard GSH:**

A standard stock solution of reduced GSH was prepared (5 mg/dl), serial diluted concentrations of GSH (2 -50 µg/dl) were prepared and treated exactly like 54 samples. A standard curve was drawn by plotting concentration of GSH against the corresponding absorbance at 412 nm (Fig.5).

**Calculation:**

Concentration of GSH in control and autistic plasma samples were obtained from the standard curve.

**Measurement of enzymatic activity of catalase**

***Chance (1954)***

Catalase was measured according to the method of Chance (1954) in which the rate of H2O2 dissociation / minute by the enzyme was followed through measuring the change in absorbance at 240nm.

**Reagents:**

- H2O2

- Sodium phosphate buffer pH 7.2

**Assay of Catalse:**

- The reaction mixture contained in a total voume of 3 ml, 1.5 ml of 0.2 M sodium phosphate buffer pH 7.2, 1.2 ml of 0.5mM hydrogen peroxide and enzyme. The reaction was started by adding H2O2 and the rate of change in absorbance was measured at 240 nm for two minutes. Values were expressed as µ moles of H2O2 dissociated / minute/ ml plasma.