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Novel biomarker for neurodegenerative diseases- motor neuron disease (MND), cerebellar ataxia (CA) and Parkinson's disease (PD)

Shivani Pandey[a,*], Babita Singh[a], Satyndra Kumar Yadav[a,b], Abbas Ali Mahdi[a]

[a] Department of Biochemistry, KGMU, Lucknow 226003, UP, India
[b] Department of Biological Sciences, Yathartha Institute, Lucknow 226003, UP, India

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ABSTRACT

Oxygen is the most mandatory component of living organism and it may at times produce highly reactive species, the free radicals, which are destructive to normal living tissues. Degenerative diseases of central nervous system (CNS) are quite common, contributing significantly to morbidity as well as mortality %. In neurodegenerative diseases such as motor neuron disease (MND), Cerebellar Ataxia (CA) and Parkinson's disease (PD), there is no direct evidence for involvement of metals and free radicals in the etiology but circumstantial evidence provides a hypothesis that alteration in metals and free radicals contribute to the pathogenesis of neurodegeneration in these disorders. The aim of the present study was to estimate free radicals cascade i.e. damage caused in terms of malondialdehyde (MDA) and defense system Superoxide dismutase (SOD) and catalase in blood and cerebro-spinal fluid (CSF) of neurodegenerative diseases (MND, CA and PD), to analyze correlation with level of free radical and the clinical variables like age, severity of diseases and duration of illness and any possibility from this clinical parameters to identify a biomarker for diagnosis of neurodegenerative diseases. The level of MDA in CSF was 0.46 ± 0.17 in case of MND, 0.49 ± 0.13 in case of CA and 0.47 ± 0.16 in case of PD as compared control group (0.22 ± 0.06) whereas in blood MDA level was 0.10 ± 0.04 in case of MND, 0.33 ± 0.41 in case of CA and 0.47 ± 0.46 in case of PD as compared control group (0.04 ± 0.03). It was found to be highly significant ($p < .001$). In CSF and blood both catalase activity was statistically significantly higher as compared to control group of all cases (MND, CA and PD) and SOD activity was statistically significantly lower as compared to control group of all cases. Free radical parameters in human CSF might be a novel biomarker for the early clinical identification of neurodegenerative diseases.

1. Introduction

Alzheimer's disease (AD), Parkinson's disease (PD), Cerebellar ataxia (CA) and Amyotrophic lateral sclerosis (ALS) and Motor Neuron disease (MND) are the major human neurodegenerative disorders. Each is an age related, progressive disorder that affects specific groups of vulnerable neurons in the central nervous system (CNS). ALS also called motor neuron disease is a progressive paralytic disorder. The paralysis is due to degeneration of large motor neurons of the brain and underlying cause of the degeneration is not known. PD is characterized by loss of nigrostriatal dopaminergic neurons in the substantia nigra pars compacta (SNc), causing reduction in striated dopamine and intra-cytoplasmic cytoskeletal inclusion called lewy bodies. CA can be the manifestation of many disorders like infections, neoplasm, degeneration, odemyelination, drugs and toxins. The etiology of this disorder is in most case unknown. It has been long recognized that oxidative stress may be important in the etiology of a variety of late onset neurodegenerative diseases [1]. Oxidative stress has been detected in a range of neurodegenerative disease, and emerging evidence from in-vitro and in-vivo disease models suggests that oxidative stress may play a role in disease pathogenesis.

Brain consumes approximately 20% total body oxygen, although it comprises < 2% of body weight. It contains large amount of polyunsaturated fatty acids, which are components of cell membranes and substrates for free radical and the chain reaction of lipid peroxidation. The brain has capacity for regeneration and is relatively deficient in protective mechanism compared to other tissue such as liver. Iron, which can promote reactions that generate free radicals, accumulates in higher concentration in brain specific regions (substantia niga, pars reticularis, red nucleus and globus pallidus). There are indirect evidences providing a free radical hypothesis for neurodegeneration in these disorders [2–4]. Among the various theories given for

* Corresponding author.
E-mail address: drshivaninbc@gmail.com (S. Pandey).

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neurodegeneration, one of which is excitatory amino acid, which states that the generation of free radicals is the cause of degenerative process. Overstimulation of glutamate receptors causes neurodegeneration and neuronal damage through a process called excitotoxicity. Excessive glutamate, or excitotoxins acting on the same glutamate receptors, overactivate glutamate receptors (specifically NMDARs), causing high levels of calcium ions (Ca^{2+}) to influx into the postsynaptic cell [5]. High Ca^{2+} concentrations activate a cascade of cell degradation processes by free radical generation involving proteases, lipases, nitric oxide synthase, and a number of enzymes that damage cell structures often to the point of cell death [6]. There is endogenous generation of reactive oxygen species (ROS) by neuro-chemical reactions like dopamine oxidation and specialized neural conduction and synaptic transmission. Malondialdehyde (MDA) is a parameter for intensity of free radical generation and enzyme superoxide dismutase (SOD) and catalase are the parameters for the effiency of cellular defense system to neutralize free radical generation. The treatment of above these disease conditions is still lacking as basic aetiopathogenesis is not clear.

The present study is being undertaken to estimate free radicals cascade i.e. damage caused in terms of malondialdehyde (MDA) and defense system superoxide dismutase and catalase in blood and cerebrospinal fluid (CSF) of neurodegenerative diseases (MND, CA and PD) and to explore any correlation with level of free radical cascade (Oxidant/Antioxidant) and the clinical variables like age, severity of diseases and duration of illness. Furthermore, any possibility from these clinical parameters, to identify a biomarker for neurodegenerative diseases.

2. Materials and methods

2.1. Participants and samples

The present study was carried out in the Department of Neurology and Department of Biochemistry, King George's Medical University, Lucknow. Study included total 82 cases of both sexes of different age groups (> 15 years to < 75 years) which comprised of-.

Control group- 20 cases, included fracture neck femur patients, hysterical hemipaertic patients and muscular dystrophy patients with age 25–70 years.

Study group- included 62 patients out of which:

➢ 24 patients were of Motor Neuron disease (MND)
➢ 18 patients were of Cerebellar Ataxia (CA)
➢ 20 patients were of Parkinson's disease (PD)

Selection of Cases: The patients suffering from neurodegenerative disease including MND, CA and PD within the age group 25–70 years, attending the neurology OPD were included in the study.

2.2. Criteria for diagnosis: the diagnosis of these disease was based on the following criteria:-

2.2.1. Motor Neuron disease (MND)

Diagnostic criteria of MND given by World Federation of Neurology Conference 1990 EL, Escorial spain which are as follows-

- Lower Motor Neuron Signs (including EMG features in clinically normal muscles)
- Upper Motor Neuron Signs
- Progression of the disorder.

2.2.2. Parkinson's disease (PD)

Patients had at least two of the following Cardinal signs-

- Bradykinesia
- Resting tremors
- Cog wheel rigidity
- Postural reflex impairment

Severity of PD includes detection scale of Hoehn and Yahr (1967) [7].

2.2.3. Cerebellar Ataxia (CA)

Progressive unremitting cerebellar ataxia, exclusion of Vascular, metabolic and other causes. Severity of CA includes Klockgether et al. (1990) method [8].

2.3. Collection of samples

5 ml blood and CSF were collected with informed consent from both groups i.e., control and study group. CSF collection was done by lumbar puncture in both groups in sterile glass vial, in which small amount of ascorbic acid (powder) was added as a preservative and sample were kept in freezer till the estimation.

2.4. Bio-chemical estimation

2.4.1. Estimation of plasma malondialdehyde (MDA)

Study was carried out on blood plasma and CSF of both groups. MDA levels were measured by thiobarbituric acid (TBA) test in which one molecule of MDA reacts with two molecules of TBA with production of a pink pigment having an absorption maximum at 532–535 nm. One ml sample was taken for estimation of MDA content. The proteins were precipitated by adding 1.0 ml of 50% trichloroacetic acid (*w/v*). The reaction mixture was cooled. The chromophore was developed by the addition of 1.0 ml of 0.67% TBA placed in boiling water for 30 min. Absorbance was determined at 535 nm in a spectrophotometer. MDA levels were expressed as nmoles MDA/ml plasma and were calculated based on extinction coefficient i.e. 153,000 for MDA-TBA adduct. It has been estimated that 99.7% of the absorbance at 535 nm which is found in standard TBA assay results from MDA and only 0.3% or less is due to all other aldehydes. Each samples was analyzed in duplicated and then mean was taken for furthers analysis. Reagent, blanks and standard were taken for each set of experiments [9].

2.4.2. Estimation of Superoxide Dismutase (SOD)

The superoxide dismutase activity was determined by the spectrophotometric method with minor modifications. The reaction mixture consisted of 0.05 M sodium carbonate buffer pH (10.2), 5 µ moles epinephrine as a substrate and a suitable amount of enzyme. CSF and Diluted hemolysate was used as enzyme preparation. The rate of adrenochrome formation was determined with/ without enzyme preparation. The SOD activity was determined for 1 min and expressed as enzyme units/mg protein. The unit of enzyme was calculated as the amount of enzyme required to inhibit 50% of the auto oxidation of epinephrine [10].

2.4.3. Estimation of catalase

Catalase activity was measured in hemolysate by the spectrophotometric method. The spectrophotometer was adjusted to 100% transmittance at 240 µm with a cuvette containing 3 ml of 50 µm phosphate buffer Ph 7.0 plus catalase enzyme preparation (hemolysate). The matching experimental cuvette contained 3 ml of buffer having approximately 40 µmoles hydrogen peroxide and aliquot of haemolysate. The absorbance was recorded for 1 min. The unit of enzyme activity was calculated on the basis of molar extinction coefficient as $0.04cm^2$/ µ mole at 240 nm H_2O_2 decomposed [11].

2.5. Statistical analysis

The data were statistically analyzed by student's 't' test for comparison of two groups with Pearson correlation coefficient, linear regression between biochemical profile and age, duration of illness,