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RESEARCH ARTICLE

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STUDY OF PROTEIN OXIDATION PRODUCTS AND ANTIOXIDANTS STATUS IN PRIMARY BRAIN TUMOR PATIENTS.

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ABSTRACT

Advanced oxidation protein product (AOPP) and protein carbonyl (PC) as protein oxidation products (PPP) and antioxidants such as reduced glutathione (GSH),total thiol (TT) and albumin levels were analyzed in 27 patients with diagnosed primary brain tumor and 25 normal healthy age and sex matched controls. Statistically significant increased (p-0.001) levels of AOPP and PC were noted; GSH and TT levels were increased (p-0.833) & (p-0.032) respectively with decreased albumin levels (p-0.629).Hence increased oxidative stress signified by increased PPP and altered antioxidant levels may have a role in etipathogenesis of primary brain tumor.

KEY WORDS

Primary brain tumor, Protein oxidation products,Antioxidants.

INTRODUCTION

Increasing number of brain tumors are being reported every year all over the world. This may be due to change in the life style and multiple etiopathogenetic factors(1).Brain tumors are responsible for 2% of all cancer deaths. These are 1/5th as common as breast and lung cancers(2).These affect all ages but manifest at 2 distinct peaks; one in childhood (3-12yrs) and another in adults (55-65 yrs).In children they constitute 2nd most common malignancy after leukemia(3).The 5 year survival rate for brain tumors are 6th lowest among all types of cancer following pancreas, liver, esophagus, lung and stomach respectively(4).For a variety of human cancers, chronic infection and inflammation have long been recognized as risk factors. It has been suggested that active oxygen species such as superoxide anion, hydrogen peroxide and hydroxyl radical generated in inflamed tissues can damage the target cells, resulting in DNA damage and being able to contribute to tumor development(5).Free radical production is universal in all respiring organisms and is enhanced by many disease processes, exposure to carcinogens and under conditions of stress(6).Growing evidence indicates that reactive oxygen species (ROS) are associated with the different steps of carcinogenesis through structural DNA damage (2).In this study we are focusing on combination of oxidative stress(protein oxidation products) and antioxidant status in relation to etiopathogenesis of primary brain tumors.

MATERIALS AND METHODS

Blood samples were collected from 27 patients diagnosed to have primary brain tumors before surgery, chemotherapy or and radiotherapy. Age and sex matched 25 healthy persons who are devoid of conditions like hypertension, diabetes mellitus, epilepsy, psychiatric disorders or history of any drug intake, smoking and alcohol consumption are selected as controls. The ethical committee of Kasturba Medical College, Mangalore approved the study. Samples were collected from following hospitals:

1. K.M.C. Hospital, Manipal,
2. KMC hospital Mangalore,
3. A.J. Medical college Hospital Mangalore,
4. Father Muller's Medical college Hospital, Mangalore.

   5ml of venous blood was collected into heparinised Vaccutainers under aseptic precautions from both patients and normal controls. The blood was centrifuged at 3000 rpm and plasma was separated into separate sterile vials.

The following parameters were estimated in our study;
Advanced oxidation protein product (AOPP) and protein carbonyl are protein oxidation products; total thiol, reduced glutathione (GSH) and albumin are antioxidants.

AOPP Was Estimated By Modified Witko's Method (7**).**
Concentrations of AOPP were expressed as mmols/L by measuring absorbance in acidic conditions at 340nm in the presence of potassium iodide (KI).

Total thiols measured in the plasma by G.L. Ellmans' procedure(8).
The sulfhydryl groups in the plasma react with 10mM 5-5′ dithiobis 2-nitrobenzoic acid (DTNB) in abosolute methanol (Ellman's reagent) to produce a yellow colored compound whose absorbance is read at 412nm.

Plasma glutathione was measured by method of Ernest Beutler(9).
Virtually all the non-protein sulphydryl group of erythrocytes is in the form of reduced glutathione. DTNB is a disulfide readily reduced by sulphydryl compounds to an intensely yellow compound. The absorbance of the reduced chromogen is measured at 412nm and is directly proportional to the glutathione concentration

Protein carbonyl content was determined by method of Levin et al (10).
Introduction of carbonyl group into amino acid residues of proteins is the hallmark for oxidative modification that is mediated by free radicals.Reaction of the carbonyl group with the 2,4-dinitrophenyl hydrazine(DNPH) forms a yellow colored 2,4-dinitrophenyl hydrazone which is measured spectrophotometrically.

Total protein (TP) and albumin (TA) was measured by Biuret method in the plasma (11).

Statistical Analysis was done using student 't' test and 'z' method.

RESULTS

1. The levels of GLUTATHIONE were increased in brain tumor patients when compared to controls with the 'p' value 0.833
2. The levels of TOTAL THIOLS were increased significantly in brain tumor patients when compared to controls with the 'p' value 0.032
3. The levels of AOPP were Very high significantly increased in brain tumor patients when compared to controls with the 'p' value 0.001
4. The levels of PROTEIN CARBONYL were increased in brain tumor patients when compared to controls with the 'p' value.0.001
5. The levels of TOTAL PROTEIN were decreased in brain tumor patients when compared to controls with the 'p' value. 0.611
6. The levels of ALBUMIN were decreased in brain tumor patients when compared to controls with the 'p' value. 0.629.
7. The levels of GLOBULIN were increased in brain tumor patients when compared to controls with the 'p' value 0.993

Table – 1

Comparison of protein oxidation products and antioxidant levels in primary brain tumor and normal controls.

PARAMETERS	MEAN±SD CONTROLS n=25	MEAN±SD TEST n=27	'p' VALUE
GLUTATHIONE (mg/dl)	10.8760±4.13112	11.378±3.25902	0.833
TOTAL THIOLS (mmols/L)	0.5472±0.17167	0.6837±0.23531	0.032 S
AOPP (mmols/L)	0.0672±0.03903	0.1231±0.05156	0.001 VHS
PROTEIN CARBONYL (µmol/L)	2.80000±0.63246	7.2926±3.39138	0.001 VHS
TOTAL PROTEIN (g/dl)	6.9220±0.66192	6.8289±0.64783	0.611
ALBUMIN (g/dl)	4.5020±0.45150	4.4370±0.50755	0.629
GLOBULIN (g/dl)	2.3752±0.61329	2.3930±0.72372	0.993

(n= number of samples/ subjects, SD= Standard Deviation, p=Test of significance, S=Significant, VHS=Very highly significant.)

DISCUSSION

Oxidative stress is believed to play a key role in tumor formation. Although this mechanism could be especially pertinent for brain tumors given the high oxygen consumption of the brain. Very little has been published regarding brain tumor risk with respect to relationship between antioxidants and oxidative stress. So, in our study we made an attempt to correlate between the antioxidant status and oxidative stress. The importance of the AOPP has been pointed out as mediator of monocyte activation state associated with chronic ureamia(12).These products may act as inflammatory mediators triggering the oxidative "ignition" of neutrophils, monocytes and T-lymphocytes, leading to upregulation and excessive stimulation of dendritic cells, suggest that the pathogenesis of disease is increased due to the Reactive oxygen species (ROS) & Reactive nitrogen species (RNS) cause oxidation or nitration of plasma proteins such as ceruloplasmin, transferrin, fibrinogen, albumin etc(13).AOPP were proposed as one of the possible markers of oxidative injury, which originates under oxidative and carbonyl stress and increase global inflammatory activity(14). Ramazan Amanvermez et al (15) while studying brain tumors have been reported an increase in the levels of AOPP. Tasarova P et al (16) also reported elevated AOPP levels in breast cancer patients.

Elevated levels of protein carbonyl group content are reported in various diseases. This present study goes in agreement with the studies done on protein carbonyl status in Brain tumor patients by Winterbourne et al(17) and M. Rajesh et al(18).The elevated levels strongly suggest that oxidative stress may play a significant role in the formation of brain tumors (19).Carbonyl formation of proteins dependent on metal ions such as Fe^{2+} & Cu^{2+}.These can bind to cat ion binding site in protein and in turn when they come in contact with H_2O_2 or O_2 they can change the side chains of amino acids to carbonyl groups(17). Accumulation of carbonyl groups on protein results in series of chemical modifications, results in the formation of protein oxidation products or advanced glycation end products(AGE)(20). AGE induces the production of cytokines, growth factors and proteases(21).In the present study we have observed a highly significant increase in both AOPP and protein carbonyls. This supports the involvement of oxidative stress in brain tumor pathology. Plasma thiols have been the object of growing interest, because numerous studies have indicated that even mild degree of hyperhomocysteinemia is associated with increased risk of developing occlusive vascular diseases (22).However, the mechanism behind the vascular injuries is still unknown.

Plasma thiols levels were increased significantly in the present study. Significant increase in the level of total thiols goes in agreement with the studies done on brain tumor patients by Bicikova marine et al(23) and wood Joon Chung et al(24). These studies showed significantly higher levels of total thiols in cases of glioblastoma multiforme and pituitary adenoma compared with other types of Brain tumors. Oxidative stress and excitotoxicity are the factors that may rise as a consequence of homocysteine levels in brain tissue (22).This may be reflected in the plasma and may be the cause for the observed increase in the total thiol levels.

Glutathione is powerful antioxidant which protect the cell against damage from free radicals and other electrofiles. Glutathione concentration increases in course of carcinogenesis to compensate the action of free radicals (25,26). Albumin also acts as cellular antioxidant which protects the neurons from toxic metabolic oxidants and during ischemia; its levels drops down during carcinogenesis and chronic illness (27).

Thus, our results of present study supports the involvement of oxidative stress particularly protein oxidation and altered antioxidant levels supporting the etiopathogenesis of primary brain tumors.

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