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Oxidative stress and brain diseases: Biomarkers and analytical methodologies

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Oxidative stress is the imbalance between production of reactive oxygen species (ROS) and ability of the biological systems to readily detoxify the reactive intermediates or to repair the resulting damage. Disturbances in the normal redox state of tissues can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell including proteins, lipids and DNA. Furthermore, some ROS can even act as messengers through a phenomenon called redox signaling. In humans, oxidative stress is involved in many pathogenic mechanisms triggering aging processes and affecting organs as liver and brain. In the present paper, the role of oxidative stress is considered in relation to three neurodegenerative pathologies those are the most common in western countries and have analogies in brain damages notwithstanding different etiologies: i) Alcohol abuse by environmental etiology, ii) Down Syndrome by genetic etiology, and iii) Alzheimer's disease by age-related etiology. Recent studies have described the results about application of different biomarkers of oxidative stress to study these brain pathologies. The present paper deals with the diagnostic applications of the oxidative-stress biomarkers like malondialdehyde, heat shock protein, ROS and retinol binding protein in clinical handling of patients and discusses their diagnostic power taking into account cost/benefit ratio too.

Keyword: Analytical procedures, biomarkers, brain diseases, oxidative stress

Introduction

Oxidative stress is the condition that occurs when the steady-state balance of pro-oxidants to antioxidants is shifted in the direction of the former, creating the potential for organic damage. Pro-oxidants are by definition *free radicals*, atoms or clusters of atoms with a single unpaired electron. Physiologic concentrations of pro-oxidants are determined both by internal and external factors. Pro-oxidant reactive oxygen species (ROS), for example, are normal products of aerobic metabolism and have important roles in cell-signalling, indeed they are continuously produced by cells as part of their metabolic processes. However, under pathological conditions ROS production can increase, surpassing the body's detoxification capacity and thus contributing to pathogenesis. The overproduction of reactive species results in oxidative stress, a combination of increased formation of oxygen derived radicals and reduced antioxidant capacity. This imbalance always results in the damage of cellular components, such as lipids, proteins and DNA, and can affect metabolism and cellular vitality up to necrosis and apoptosis. External sources of free radicals include exposure to environmental toxics, such as, ionizing radiation, ozone, nitrous oxide, cigarette smoke including passive inhalation and heavy metals. Heavy alcohol drinking, dietary intake of unsaturated fat and other chemicals, and compounds present in food and water may also trigger oxidative stress[1].

Antioxidants are chemical compounds that can bind to free radicals and thus prevent them from damaging healthy cells. Antioxidants can be divided into enzymatic and non-enzymatic subtypes. Several antioxidant enzymes are produced by the body and the three major classes are catalase, glutathione (GSH) peroxidases and superoxide dismutases (SODs). Non-enzymatic antioxidants include the innate compound glutathione and antioxidant vitamins obtained through the diet, such as, α-tocopherol (Vit E), ascorbic acid (Vit C), retinol (Vit A) and β-carotene[2]. In humans, oxidative stress is involved in many diseases and in many pathogenic mechanisms affecting brain function. The present paper considers three brain pathologies representative of three different main etiologies: alcohol abuse/alcohol-related dementia as environmental disease, Down's syndrome as genetic disease, and Alzheimer's disease

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as age-related diseases. For all these disorders, many studies have indicated that oxidative stress has a pivotal role in their pathogenesis. In fact, investigations of Down's syndrome, Alzheimer's disease, heavy drinker's brains and non-neuronal tissues have demonstrated many signs of ROS attack, e.g., lipid peroxidation and protein and DNA oxidation, besides mitochondrial abnormalities.

Till today, the assessment of oxidative stress in clinical settings has traditionally been hampered by the use of different assays and techniques that gave some conflicting results. Furthermore, different biomarkers are used to determine oxidative stress as a result of increased oxidation or antioxidants deficiency. In the first case, we can detect in biological fluids reactive oxygen metabolites (ROMs), products of lipid peroxidation, such as, malondialdehyde (MDA), and protein and DNA oxidation. In the second case, we can measure enzymatic antioxidants defence by determination of activities of catalase, glutathione peroxidase (GPx) and Superoxido dismutase (SOD). Non enzymatic way may be determined by measurement of α-tocopherol (Vit E), retinol (Vit A), retinol-binding-protein (RBP), heat shock protein (HSP) and ascorbic acid (Vit C)[3]. Oxidative stress may be also indirectly assessed by total capacity for antioxidant defense that includes enzymatic and non enzymatic antioxidant activity and antioxidant status, i.e., ability of blood antioxidants to neutralize a pro-oxidant compound *in vitro*. The most used analytical techniques include gas chromatography (GC), high performance liquid chromatography (HPLC), electrophoresis and ELISA. For direct detection of free radicals, the unique technique is electron spin resonance. The present paper deals with three typical neurodegenerative conditions where oxidative stress plays a pivotal role and shows the diagnostic applications of the oxidative-stress biomarkers malondialdehyde, heat shock protein, ROS and retinol binding protein. Their use in clinical practice is also discussed in consideration of the problems related to analytical methodologies currently used for their determination

Alcohol Abuse and Oxidative Stress

Alcohol abuse is responsible for heavy health and social problems worldwide. Alcohol drinking is the most socially-accepted addictive drug and a common feature of social gatherings. Probably for this reason the risks related to heavy drinking are largely underestimated, above all in particular population as teen agers, pregnant women and elderly people. Heavy drinking may affect every organ and body district triggering coronary-heart disease, neuro-degenerative disease, chronic diseases and cancer. Furthermore, behavioural effects of alcohol abuse strongly affect social life inducing violence, antisocial and at-risk behaviours[5]. Genetic susceptibility plays a pivotal role in the development of "*alcohol disease*" that represents a typical example of interaction between environmental and individual genetic factors. In fact, ethanol-induced toxic effects show a huge inter-individual variability mainly due to differences in alcohol metabolism. Alcohol metabolism depends on the genetic polymorphisms of the involved enzymes and their induction due to ethanol itself[6]. After ingestion, ethanol is rapidly absorbed by the gastrointestinal tract with a rate varying with timing, dosage and drinking pattern in addition to the nutrition status of the exposed subject. Due to its water solubility, ethanol is readily and uniformly distributed to the water space of body. Elimination of ethanol takes place mainly through metabolic biotransformation (95-98%), while small fractions are detected in breath (0.7%), sweat (0.1%) and urine (0.3%)[7]. More than 90% of the ingested dose in humans undergoes liver metabolic oxidative and non-oxidative biotransformation.

The main enzymatic way of ethanol metabolism involves alcohol dehydrogenase (ADH) with the production of acetaldehyde, a highly toxic metabolite, which is metabolized by the mitochondrial aldehyde dehydrogenase (ALDH) to acetate, and then to CO_2 and water. Acetaldehyde is normally present within the body at micromolar concentrations. Nevertheless, it can promote cell death by depleting glutathione levels and inducing oxidative damage. Acetaldehyde binds to specific amino acid residues on the structural and functional proteins to form acetaldehyde-protein adducts, causing a pathological immune response[8].

The second way of ethanol metabolism is cytochrome CYP2E1 that converts ethanol to acetaldehyde, which is oxidized and releases oxygen-derived free radicals. Hepatic CYP2E1 shows much lower affinity for ethanol than ADH and it is responsible for only a limited amount (about 10%) of ethanol oxidation to acetaldehyde in cases of moderate alcohol intake. When ADH is saturated because of alcohol abuse, CYP2E1 contribution

becomes relevant since CYP2E1 is inducible by ethanol itself[9]. CYP2E1 increases the rate of ethanol clearance and contributes to the metabolic tolerance seen in the alcoholics[10]. It is expressed mainly in liver and in other organs including the central nervous system, and plays an important role in brain disease. The increased rate of CYP2E1 oxidative activity leads to the formation of ROS and ethanol-derived (hydroxyethyl) free radicals, and can thus initiate lipid peroxidation with various breakdown products. One of these is malondialdehyde, which is considered a reliable marker of oxidative stress. Alcohol drinking also increases the circulating oxidized low density lipoproteins. The source of these extracellular oxidized lipid species is assumed to reflect hepatocyte production of O_2 by cythochrome CYP2E1. Alcohol related liver disease is associated, in particular, to mithocondrial oxidative stress with specific mitochondrial damage. This was supported by morphological evidences of disrupted mitocondria and increased mitochondrial aspartato transaminase (mAST) in the serum[11]. Various polymorphic genes coding for enzymes involved in ethanol metabolism may act as a protective/enhancing factor towards alcohol abuse and alcohol-related pathologies[12].

Alcohol toxicity is both due to ethanol per se and to its metabolic products including ROS. Toxic action is mediated mainly by acetaldehyde and other metabolites like fatty acid ethyl esters (FAEEs): ethyllinoleate, ethylpalmitate and ethyloleate. Acetaldehyde has the main role in developing alcohol diseases together with free radicals produced during ethanol metabolism. FAEEs are also considered very important in alcohol-related damage. In particular, FAEEs seem to contribute to physical and behavioural damages, globally defined as foetal alcohol spectrum disorders (FASD) that affect the child of drinking mother during pregnancy. For this reason, the determination of FAEEs in the mother's fluids and in meconium of the newborn is considered one of the most important markers of prenatal alcohol exposure[13]. To study the ROS during pregnancy and their implication in human placentation and related complications is a relatively recent field but it is expanding rapidly. ROS seems to play a central role in many signal transduction pathways and oxidative stress has been implicated in preterm premature rupture of the membranes, a condition associated with proteolytic degradation of the collagen fibres in the chorio-amnion. Increased ROS may arise from many factors including infection and inflammation, cigarette smoking and alcohol use[14]. All enzymes involved in ethanol metabolism have been shown to be polymorphic, so recent studies have looked in for a correlation between metabolic variability and differences in alcohol related effects. The genetic polymorphisms of ADH and ALDH are involved in the susceptibility to alcohol dependence and alcohol-related diseases. The degree of polymorphism depends on racial and ethnic groups. Allelic variants of cytochrome CYP2E1 may affect the development of alcohol diseases as it has been suggested by animal models and molecular epidemiological studies[12]. Oxidative stress products generated by CYP2E1 participate to adverse effects of alcohol exposure by promoting lipid peroxidation and severe damages to DNA and proteins. The imbalance between ROS production and activity of antioxidants is associated with numerous deleterious consequences for the cell (lipid peroxidation or even cell death), and may play a significant role in the development of alcohol related disease (ARD). In a nutshell, alcohol-induced oxidative stress is the result of many processes that operate concurrently and trigger a lot of diseases including pregnancy complications and foetal disorders.

Biomarkers of Alcohol Abuse

Notwithstanding a lot of paper about alcohol biomarkers, the question about diagnostic power of each biomarker remains open. Some markers distinguish between effects due to long-term alcohol use, such as, γ-glutamyl transferase (GGT), carbohydrate-deficient transferrin (CDT) and mean corpuscular volume (MCV), which require several weeks or months of sustained alcohol consumption to be significantly elevated. Other biomarkers are able to demonstrate short-term alcohol use, such as, blood alcohol and acetaldehyde, 5-hydroxytryptophol and ethyl glucuronide, those are measurable soon or only few days after intake[16]. Furthermore, some dietary factors may concur in alcohol damage as happens in the case of the of Vit B1 deficiency. This alcohol-related condition is responsible for brain damage and, in particular, of Wernike-Korsakoff syndrome. So, Vit B1 deficiency may be considered not only a marker of alcohol abuse but also a prognostic index of alcohol related brain disease[17]. Clinicians can use several biomarkers to objectively assess patients' current or past alcohol use and to reveal alcohol related effects, e.g., evidence of

recent alcohol use may be relevant in monitoring compliance or relapse during treatment programmes, otherwise to reveal any use, remote or acute, is important as in the case of diagnosis of prenatal alcohol exposure[5]. Cost effectiveness is also a critical consideration in the use of biomarkers. For instance, to detect prenatal alcohol exposure, a biomarker with high sensitivity but low specificity could be used as an initial screening tool for mother and child, and a more specific and probably more expensive second-stage diagnostic tool could be employed for ultimate identification of exposed persons[18]. Several alcohol biomarkers have been used for many years and they are defined as trait markers and state markers[19]. Trait markers are biochemical markers that reveal something about a person's inherited risk of abusing alcohol and provide information about the individual genetic vulnerability toward alcohol dependence. Trait markers are connected to brain functioning as regards the level of the neurotransmitters γ-amino butyric acid (GABA), dopamine, serotonin and β-endorphin, and they are also connected to ethanol metabolism in terms of polymorphisms of ADH, ALDH, CYP2E1 and GST[12]. State markers are biochemical measures that tell clinicians something about people's recent drinking patterns, including whether they have a history of heavy drinking and whether they have had a recent binge or even just a few drinks.

Oxidative stress biomarkers are state markers used to study and evaluate the etiopathogenesis and the progression of several alcohol diseases. Lipids are the class of biomolecules most involved in oxidative stress. Lipid oxidation produces mainly aldehydes that exacerbate oxidative damage by interacting with nucleic acids and proteins and damaging mechanisms of cell functionality. Malondialdehyde (MDA) is the main and the most studied product of polyunsaturated fatty acid peroxidation. Since the 1960s several methods have been developed to assess this molecule for quantifying oxidative stress *in vivo* and *in vitro*[20]. MDA interacts with nucleic acid bases to form several different adducts. This potentially genotoxic activity of MDA may lead to mutations and subsequently to cancer. MDA toxicity is directed also towards cardiovascular stability, since it reacts *in vivo* with primary amines to form the N3-(2-propenal-lysine) and generates lysine-lysine cross-links with 1-amino-3-iminopropene and pyridyledihydropyridine type bridges. These reaction products have been detected in apoB fractions of oxidized lipoproteins (LDL) and are probably involved in the impaired interaction of the modified lipoproteins and macrophages, i.e., the phenomenon that is the basis of atherogenicity. Even if the nature of the cross-link is not yet completely determined, the inter-molecular cross-linking of collagen through MDA may significantly contribute to the stiffening of cardiovascular tissue. In the last 20 years, MDA has been recognized as a relevant lipid peroxidation marker and, in recent years, has been widely utilized in the handling of several diseases. Liver diseases have been related to oxidative stress, since plasma MDA concentrations in alcoholics with or without cirrhosis and in patients with viral cirrhosis were higher than in matched healthy controls. High MDA content has been shown in Alzheimer's type dementia patients, Parkinson's disease and results correlated with severity of alcohol withdrawal symptoms[21-22]. Adducts of MDA and DNA have been observed in pathologies or in relation to particular dietary habits. Consumption of some vegetables and wholemeal bread was inversely associated with adducts concentration, whereas alcohol and meat consumption tended to increase it. So MDA may be considered as a good biomarker to reveal and to monitor oxidative stress trigged by alcohol abuse. Oxidative stress in clinical alcohol research can be evaluated also by the determination of reactive oxidative metabolites (ROMs)[23] but today MDA determination in plasma by high performance liquid chromatographic HPLC-VIS is one of the most reliable tool to study oxidative stress in alcoholics. HPLC procedure involves alkaline hydrolysis of plasma, TBA reaction and n-butanol extraction steps[24]. Fig. 1 shows a HPLC chromatogram of MDA in the plasma of alcoholic patient. The TBA method is widely used to assess peroxidation in the whole organism and to quantify the level of oxidative stress *in vivo* and *in vitro*. Many studies demonstrated the diagnostic power of MDA determination but a crucial

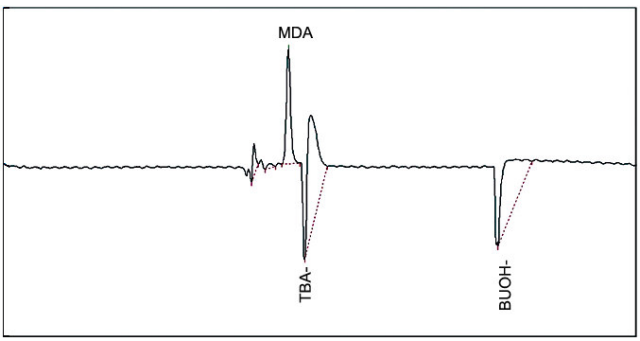

Fig. 1—Chromatogram of MDA in plasma of alcoholic patient

point of contest is the choice of reference value. In fact, there are some differences in analytical procedures that make the reference values inconsistent; certain detection methods fixed healthy plasma MDA values approximately in the range 0-1 mmol/L, while other studies report the range 4-5 mmol/L. This raises some doubts about the biological significance of the data[20]. Till today, the unique solution to this problem is to analyze every time controls and cases simultaneously by the established procedure. Optimization of analytical procedures will make MDA determination a more reliable method to be used in handling of alcohol related problems and the results will be more comparable than what it is today.

Oxidative Stress in Down Syndrome and Alzheimer's Disease

As mentioned above, oxidative stress is involved in different brain diseases that, even generated by different aetiologies, present similarities in pathological mechanisms. A relationship between the neuro-disorders, Down syndrome (DS) and Alzheimer's disease (AD) has been observed for many years. In particular, people with DS always develop the neuropathology associated with AD and often develop clinical dementia as well. Similarly, chronic alcohol abusers tend to develop Alzheimer's like dementia[25]. Researches on the molecular event surrounding the pathology of AD and DS support the specificity of this relationship. Besides alcoholics, oxidative stress occurs in individuals with DS and those develop AD. It can be due to abnormal mitochondrial energy generation and folate metabolism, early endosome abnormalities, abnormalities in amyloid precursor protein (APP) metabolism and loss of functional cholinergic neurons. Evidence for the neuropathological association of DS and AD was first published by Jervis in 1948. People with DS, due to full trisomy of chromosome 21, develop the neuropathology seen in AD and no other syndrome splits this feature with DS. The commonly accepted hypothesis is that the appearance of amyloid plaques in DS is due to the presence of gene encoding the amyloid precursor protein (APP) on chromosome 21. This leads to overexpression of APP and therefore overproduction of β-amyloid protein, which is the major component of amyloid plaques. There are many genes on chromosome 21 that are important for metabolic systems. Oxidative stress is due to ROS, mitochondrial energy metabolism (MT/ROS), transulfuration/one-carbon (TS-1C) metabolism and cholesterol metabolism; these have been hypothesised to play a role in DS and AD. ROS are produced as a consequence of metabolism and it is estimated that up to 2% of oxygen used for intermediary metabolism is converted to ROS[26]. The mitochondrium is the site of oxidative phosphorylation and energy generation, and generates the majority of ROS in mammals. Over the past 12 years, a wide variety of neurodegenerative diseases has been linked to the mutations in mitochondrial genes located in either the mitochondrial DNA (mDNA) or the nuclear DNA (nDNA). These disorders encompass an array of inheritance patterns and a plethora of symptoms ranging from lethal neonatal multi-symptom disorders to the later onset of myopathies, cardiomyopathies, movement disorders and dementia. The basis for the genetic and phenotypic variability of mitochondrial diseases lies in the multiplicity of the mitochondrial genes dispersed across the human genome and the variety of cellular pathways and functions in which the mitochondria play a central role[27]. The brain is particularly sensitive to ROS-induced damage because it generates and uses about 20% of the energy in a human and the brain contains a large amount of unsaturated fatty acids, which are sensitive to ROS-induced damage[26]. Some researchers consider oxidative stress one of the earliest events in pathogenesis of damages in the AD and DS population. Aggregation of hyperphosphorylated tau is one of the characteristics of neuropathological lesions of AD and other neurodegenerative disorders. In the initial phase of AD disease development, β-amyloid deposition and hyperphosphorylated tau aggregation may function as compensatory responses and downstream adaptations to ensure that neuronal cells do not succumb to oxidative injuries. During the progression of the disease, the antioxidant activity of both agents evolves into pro-oxidant activity representing a typical gain-of-function transformation, which can result from an increase in reactive species and a decrease in clearance mechanisms[28]. The chronological sequence of pathological events in DS shows the relationship between oxidative stress and amyloid-β (Abeta) deposition. Recent studies suggest that, in brains of patients with DS, increased levels of oxidative damage occur prior to the onset of Abeta deposition[29].

Biomarkers of Oxidative Stress in Down Syndrome and Alzheimer's Disease

Several studies have reported that mitochondrial function and ROS metabolism are altered in neurons and astrocytes cultured from foetuses with DS and this alteration is associated with altered APP metabolism. Isoprostanes markers of lipid peroxidation are elevated in the brains of individuals with DS and AD. The levels of isoprostanes are also elevated in the urine of a person with DS, and the levels also increase with age. The isoprostane, 8,12-iso-iPF2α-VI is a specific marker of lipid peroxidation. These results suggest that increased *in vivo* lipid peroxidation is a prominent component in the early course of Down syndrome[30].

The frequently used techniques for determination of isoprostanes are GC/MS and HPLC-MS/MS. APP metabolism has been linked specifically to ROS metabolism and recent studies suggest that a function of APP may be to produce low levels of 7-hydroxycholesterol. Higher levels produced by Abeta could contribute to the oxidative stress and cell loss observed in AD. The metabolism of Abeta itself can produce oxidative stress[31]. Furthermore, Abeta has been found to cause aberrant mitochondrial metabolism through binding to the mitochondrial protein, called amyloid-binding alcohol dehydrogenase (ABAD) that inhibits its activity. So ABAD is a direct molecular link from Abeta to mitochondrial toxicity and this interaction has been hypothesised to directly contribute to oxidative stress in AD[32]. Moreover, oxidation of Abeta itself has been hypothesised to be an early event in amyloid plaque biogenesis[33]. Probably, there is a close relation between the APP metabolism associated with AD and DS, and the abnormal oxidative stress. It is important to understand the role of oxidative stress and its relationship to Abeta metabolism, since this may lead to new therapeutic approaches to AD and probably to DS. At least 17 genes involved in energy and ROS metabolism, and potentially in APP metabolism, are located on chromosome 21—mitochondrial ribosomal protein L39, ATP synthase FO coupling factor 6, NF-E2 related factor (NRF2), Bach1, amyloid precursor protein (APP), cytosolic superoxidase dismutase (SOD1), phosphoribosylgycineamide transformilase (GART), NADPH [quinine reductae-like (CRYZL1), ATP synthase OSCP subunit, mitochondrial ribosomal protein S6, calcipressin 1 (ADAPT78), carbonyl reductase 1 (CBR1), carbonyl reductase 3 (CBR3) and thioredoxin-like protein (SH3BGR)], mitochondrial NADH [oxidoreductase 10kDa subunit (NDUFV3), cystathionine beta synthase (CBS) and C21orf2 mitochondrial protein]. Many signalling pathways have been predicted to play roles in both AD and DS. In several cases, genes on human chromosome 21 participate in these pathways, as well as genes on other chromosomes. This means that system biology and computational biology methods are important to understand how these biological pathways interact[34]. To investigate oxidative stress in DS and AD subjects, protein expression patterns can be explored using a proteomic approach. The goal would be to examine the protein spectrum and its biological functions to detect diagnostic markers and novel drug targets. A proteomic analysis comprises two steps: separation of protein mixtures by two-dimensional (2-D) gel electrophoresys and identification of the separated proteins by various analytical methods mainly by HPLC-MS. The 2-D gel electrophoresis consists of two-steps separation in two dimensions: first the separation of proteins on the basis of differences in their net charge by isoelectric focusing (IEF) and then separation of the focused proteins on the bases of different molecular masses by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Proteomic researches improve the knowledge of concentration, modification and interaction of proteins that are fundamental in determining the phenotype of living organism. Plasma and serum are very precious source for proteomic researches because they are easy to obtain from patients and contain 60-80 mg/mL of proteins. Many of these proteins have largely been used for many years as biomarkers of diseases and indicators of physiological functions. The development of plasma proteome studies promises significant advances in biological and clinical research. Mono- and two-dimensional polyacrylamide gel electrophoresis (PAGE) are considered as primary tool in separating thousand of plasma proteins (Figs 2 & 3) to compare control and case samples, and to reveal differently expressed proteins[35]. Assay of antioxidant systems or heat shock proteins may indicate prior exposure of the cell to oxidative stress. Retinol is the major circulating form of Vit A and it has shown to have some antioxidant properties. But recent studies have demonstrated that it is a prooxidant at higher doses and modulates antioxidant enzyme activity. Anyway, the mechanism by which

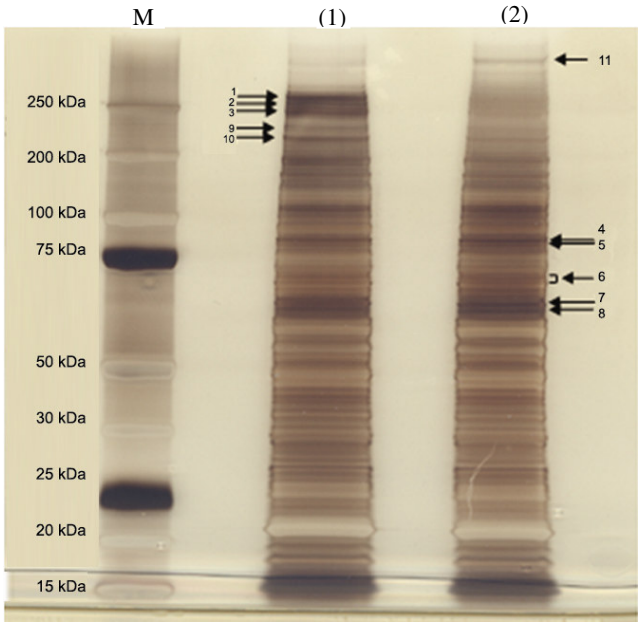

Fig. 2—Mono-dimensional electrophoresis gel of serum: M, Standard BSA; 1, Healthy subject; 2, DS patient showing over expression of hsp70 and hsp90.

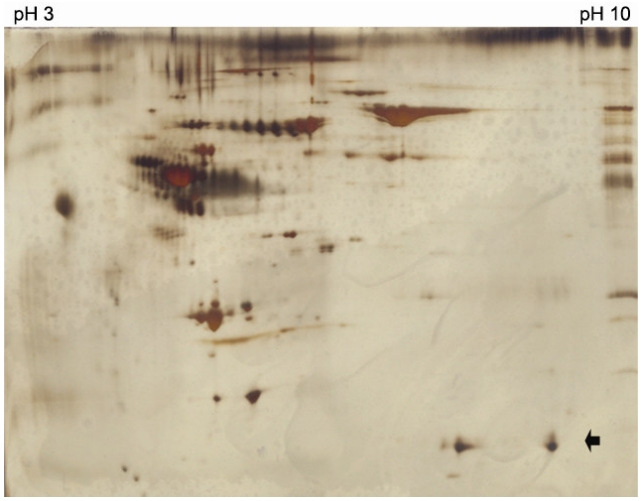

Fig.3—Bi-dimensional electrophoresis gel of serum from DS patient: Right lower section shows over expression of RBP

retinol can act as a prooxidant is not well elucidated yet[36]. Vit A plays an essential role in maintaining mammalian health. It is required for many crucial biological functions, such as vision, reproduction, growth and immunity. Two oxidative enzymatic reactions convert retinol first to retinaldehyde and then to retinoic acid. Vit A is normally transported in plasma as retinol linked by a specific transport protein known as retinol binding protein (RBP). When dietary Vit A is not available, RBP is able to mobilize retinol from Vit A stores in the liver to supply peripheral cells and tissues with retinoids needed for various biological functions[37]. Alteration in the activity of RBP, the carrier protein for Vit A in plasma, is a reliable marker of impaired antioxidant activity. Heat shock proteins such as hsp70 and hsp90 are intracellular proteins induced by various stimuli including heat shock, hyperoxia and oxidative stress. They represent a powerful endogenous protective mechanism against free radicals in many pathological conditions[38]. In particular, hsp70 can protect cells from thermal or oxidative stress that damages proteins causing partial unfolding and possible aggregation. Hsp70 prevents these partially-denatured proteins from aggregating and allows them to refold by temporarily binding to hydrophobic residues exposed by stress. Proteomic research has been successfully applied in the investigation of the human, rat and mouse brains. Protein alterations in disorders of the human central nervous system, such as, AD and DS , have been studied to provide information about gene products involved in these diseases. The recent analytical methods have made the studies about brain disease more productive and the significant results obtained are enhancing our knowledge in this field[39-40]. Unfortunately, many problems related to analytical methods are not completely solved and this make the results directly incomparable. Standardization of methods and analytical procedures will significantly improve a better handling of patients.

Conclusion

Since free radicals are unstable and difficult to measure, traditional biomarkers of oxidative stress include downstream markers of oxidative damage to macromolecules, such as lipids, proteins and DNA. The difficulty is to choose the best marker considering not only its diagnostic sensitivity and specificity but also the analytical reliability of its determination for *in vivo* studies. As a general rule, it is now accepted that techniques successfully applied to *in vitro* systems are not always equally reliable in complex biological systems. Nevertheless, there are a lot of oxidative stress biomakers that have been demonstrated reliable enough to be used in clinical practice. This is the case of MDA, hsps, ROS and RBP, those have been demonstrated effective for the diagnosis and monitoring of brain disease, such as, alcohol disease, DS and AD. Their detection by different analytical methodologies has contributed to elucidate the pathological mechanisms of these diseases. Furthermore, knowledge obtained by these

markers may facilitate the development of antioxidant intervention strategies leading to reduction in the damages due to oxidative stress. Such strategies may delay age-related degenerative diseases and enhance the quality of life particularly in the later years. The main problem that restricts the use of these biomarkers is the complexity and variety of analytical procedures that are quite expensive in terms of reagents, instrumentation and operator's training. There are yet also problems in analytical standardization, so it's difficult to compare results and establish reference values. It is hoped that improvement in analytical techniques employed in the studies will allow a larger use of these markers in the clinical practice.

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