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REVIEW

Neuromarkers and unconventional biological fluids

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Abstract

There is a growing evidence on the use of biomarkers in daily practice both as of markers of brain/multiorgan damage and/or trophic factors. However, among different tools, Activin A, S100B protein, and Hemeoxygenase-1 (HO-1 or Heat Shock Protein 32, HSP32) assessment offer the possibility to investigate brain/multiorgan function and development. This could be especially useful in perinatal medicine that requires even more noninvasive techniques to fulfill the minimal handling diagnostic and therapeutic strategy. In this regard, among different biological fluids, human milk for its unique composition can constitute a wide source of knowledge useful both in clinical daily practice and in research.

Therefore, this mini-review reports recent data on the presence and the usefulness of Activin A, S100B protein, and HO-1/HSP32 assessment in human milk as brain/multiorgan development markers. Results open up a new cue on the use of these markers in perinatal medicine as a key protein for investigations focusing on fetal/neonatal development.

Keywords: *S100B, activin A, HO-1, HSP32, brain development, milk, cardiovascular, oxidative stress*

Abbreviations: *CNS, central nervous system; CO, carbon monoxide; CSF, cerebrospinal fluid; HO-1, hemeoxygenase-1; HSP32, heat shock protein 32; RAGE, receptor advanced glycation end products; TGF, transforming growth factor*

Introduction

The term *unconventional biological fluid* refers to all conditions in which a standard laboratory parameter is not assessed in cerebrospinal (CSF) and blood fluids or whether not included in manufacturers' instructions [1]. In the last decade, especially in perinatal medicine the gold standard for sick babies monitoring was the *so-called* minimal handling to reduce sampling stress and invasive procedures [2]. In this setting, technological improvement allowed to reduce blood volume at sampling and several laboratory parameters can be assessed now through noninvasive procedures (i.e. transcutaneous oxygen and carbon dioxide partial pressures, bilirubinemia, etc.). However, unconventional biological fluids usefulness is not restricted to laboratory assessment but can be extended to clinical daily practice and treatment. These issues can be of particular interest especially in sick infants in which urine and saliva fluids have been used for hemodynamic, brain and oxidative stress biomarkers assessment [3–5]. Among noninvasive biological fluids human milk has been reported for its unique properties since it contains new markers involved in a cascade of events leading to brain, cardiac and vascular development/damage [6–9]. It is noteworthy that human milk at different periods of maturation was rich of neuro-oxidative stress biomarkers and that their concentration was higher up to 20 times more than that detected in conventional biological fluids [6–9].

Therefore, this minireview is aimed at investigating the role in human milk of some selected biomarkers such as:

1. well-established brain constituents namely Activin A and S100B protein and,
2. oxidative stress factors namely heat shock protein (hemeoxygenase-1, HO-1 or Heat shock Protein 32, HSP32).

Herein will be reported experimental and clinical findings suggestive of new biological role of neuronal and calcium binding proteins and of HSP.

Brain constituents

Activin A

Activin A is a growth factor composed of two betaA subunits belonging to the transforming growth factor beta (TGF-*beta*) superfamily of dimeric proteins. The biological activity of activin A is mediated by: (i) two different receptors types namely type I (ARI and ARIB) and type II (ARII and ARIIB), and (ii) two activin-binding proteins

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such as follistatin and follistatin-related gene. These factors bind to activin A and thereby inhibit its biological effects [3,10].

Activin A, its receptors, and binding proteins are widely distributed throughout the brain. Studies in experimental model and in humans of acute brain injury strongly correlate enhanced activin A expression as a common response to acute neuronal damage of various origins. Hypoxic/ischemic injury, mechanical irritation, and chemical damage of brain evoke a strong upregulation of activin A. Because activin A induction occurs early after brain injury, its measurement may provide a potential biochemical index of the presence, location, and extent of brain injury [3,10–12]. Of note, subsequent experimental studies have shown that activin A has a beneficial role to neuronal recovery and that, by activating different pathways, activin A exerts neuroprotective activities as well as modulates cellular and tissues growth and differentiation [3,13,14]. On the basis of the present findings, Florio et al. [9] investigated whether activin A and follistatin as neuroprotective agents were detectable in human milk.

Milk samples were collected at 3, 5, and 30 days after delivery, and activin A and follistatin were measured by using a specific and sensitive two-site ELISAs. Breast milk was obtained from a group of healthy pregnant women (27–35 years old) who delivered at term of gestation (38–42 weeks) subdivided into two groups according to delivery mode (vaginal delivery or elective cesarean section).

Results showed for the first time the presence of immunoreactive activin A and follistatin in human milk. No significant differences in concentration between the third and the fifth day after delivery was found as well as no difference of activin A and follistatin concentration between the whole and the skim milk or between spontaneous delivery and cesarean section. Milk activin A and follistatin concentrations progressively decreased from colustrum to mature milk. Of note, activin A and follistatin concentrations were significantly higher than those detected in conventional biological fluids [11–14]. The explanation of this findings may reside in: (i) activin A and follistatin trophic activity on breast tissue modulating growth and differentiation; (ii) immune function since activin A increases cytokine production from monocytes in normal humans peripheral blood mononuclear cells, exerting both pro- and anti-inflammatory effects on human tissues of pregnancy, and regulates T cell development; (iii) hormone/growth factors action on the differentiation and maturation of mammary epithelial cells during lactation, and (iv) activin A releasing in the neonatal circulation, suggesting an involvement in the regulation of growth/function of various neonatal tissues including brain [3,10].

S100B protein

S100B protein is an acidic calcium-binding protein of the EF-hand family, characterized by the most common calcium-binding motif with a helix-loop-helix structure [4] concentrated in the nervous system (CNS), where it is located in glial and neuronal cells. It is present extracellularly, intracellularly, and in the cytosol; its half-life is approximately 1-h, and it is mainly eliminated by the kidney [4]. The protein (beta-beta dimer) is present in CNS and is concentrated in the glial cells, astrocytes, Schwann cells, and neurons. It regulates several cellular functions (cell–cell communication, cell growth, cell structure, energy metabolism, contraction, and intracellular signal transduction). Elevated S100B levels are found in biological fluids, including CSF, blood, amniotic fluid, urine and recently in saliva in patients with brain damage [4,15–20]. Among several S100B functions, to date still matter of investigation, a series of clinical and experimental studies suggest that the protein acts as a cytokine with a neurotrophic effect at nanomolar concentrations, while it could be neurotoxic at higher (micromolar) concentrations [4]. Therefore, even more interesting perspectives are offered by the detection of S100B in breast milk, candidating the protein to participate in the biochemical communication between mother and newborn growth. Results in a series of studies conducted both in experimental model and in human support this issue. In details S100B levels in human milk were as follows: (i) 200 times higher than in CSF, blood, urine, and saliva [15–20]; (ii) positively correlated with the grade of maturation; (iii) higher when compared to other mammalian species (i.e. human, cow, goat, donkey, and sheep) [4,6,7,21].

Recently, studies investigated the impact of industrial preparation phases (skimmed cow milk, protein sources supplementation, pasteurization, and spray-drying procedures) on S100B. Results showed that S100B was thermostable to pasteurization procedure, but not at spry-drying phase suggesting that new procedure in preterm and term infants milks are requested to preserve protein and other potential brain constituents during industrial preparation [22].

Finally, apart S100B trophic role, similarly to previously reported [4] on S100B's role in the mechanisms of neuronal death and apoptosis, in experimental model, S100B has been found to participate in the cascade of events leading to postmyocardial infarction ventricular remodeling as follows: the transfection of a full-length cDNA of receptor advanced glycation end products (RAGE) or a dominant-negative mutant of RAGE resulted in increased or attenuated S100B-induced myocyte apoptosis, respectively. Inhibition of ERK1/2 by U0126/PD-98059 or overexpression of a dominant negative p53 comparably inhibited S100B-induced myocyte apoptosis. Therefore, it has been suggested that the interaction of RAGE and its ligand S100B after myocardial infarction may play a role in myocyte apoptosis by activating ERK1/2 and p53 signaling [23]. On this light, it can be hypothesized that authors demonstrated one of the main S100B aspects related with protein's toxicity whether detected at highest concentrations in the extracellular space. On the other hand, bearing in mind the highest S100B amount in human milk, the protein's absorption from intestinal tract trough a mechanism RAGE-mediated it can be speculated that in cardiac tissue identically to CNS the protein at physiological (nanomolar) concentrations exerts a trophic role while at elevated (micromolar) concentrations it is toxic and participate at the mechanism involved in cell death and apoptosis. Further investigations are eagerly awaited in order to clarify this issue.

Heme-oxygenase-1 (HO-1, HSP32)

Heme oxygenase isoforms catalyze the conversion of heme to carbon monoxide (CO) and biliverdin/bilirubin with a concurrent release of iron, which can drive the synthesis of ferritin for iron sequestration [24,25]. The products of the HO-catalyzed reaction, particularly CO and biliverdin/bilirubin have been shown to exert protective effects in several organs against oxidative and other noxious stimuli. In this context, it is interesting to note that induction of

HO-1/HSP32 expression by means of natural compounds contributes to protection against liver damage in various experimental models [24–26]. HO-1/HSP32 is the inducible isoform and its synthesis may be induced under various pathological conditions or pharmacological agents, whereas HO-2 is the constitutive isoform and is widely distributed in different cell type. Both isoforms are resident in the endoplasmic reticulum; however, under certain conditions, it has been shown that HO-1/HSP32 may translocate into different cellular compartments such as nuclei [27] and mitochondria [28]. Therefore, bearing in mind: (i) the unique role of human milk in infant feeding as regards a reduced risk of cardiovascular disease, diabetes, obesity, and cancer [29] because of its antioxidant properties; (ii) the importance of breastfeeding in the first months of life, when it assures protection against infectious diseases, stimulating the development of the immune system [30] (release of immunoglobulin A, antimicrobial factors, cytokines, chemokines, and growth factors [31,32]; (iii) the presence in human milk of several proteins and antioxidant enzymes (i.e. GSH peroxidase and superoxide dismutase) [18,19]; (iv) the presence of HSP in biological fluids related with the capacity of binding to specific cell surface receptors such as CD91 [33]. All together, Li Volti et al. [8] investigated the pattern of HO-1/HSP32 protein over a time course in human (from colostrum to mature milk) and milk-formulae milks by HO-1/HSP32 assessment in milks together with a computational approach. Results showed that HO-1/HSP32 is present in human milk at different maturation stages prompting authors to hypothesize that the HO system may have a major role beyond heme catabolism [6]. In particular, molecular modeling approach provided the possibility to demonstrate that HO-1/HSP32 may bind to specific receptors (CD91) in the extracellular space playing a major role in the immune system regulation.

Conclusions

The present data provide evidence that, in perinatal medicine, the assessment of novel biomarkers is becoming feasible offering a wide range of informations to physicians. This refers to the possibility of longitudinal monitoring of CNS trough the assessment in noninvasive/unconventional biological fluid such as urine and saliva. The advantages reside in a early detection of high-risk cases for brain, cardiac, and multiorgan failure [23,34] also offering the possibility to monitor the effectiveness of therapeutic strategies commonly adopted in perinatal care areas. This holds for antenatal treatments (i.e. glucocorticoids, antihypertensive and antidepressant drugs, and nitric oxide donors), for NICUs therapeutic strategies (i.e. glucocorticoids, sedative, and inotrope) and for invasive procedures (i.e. mechanical ventilation and brain cooling) [3,5,10,35]. Among therapeutic protocols, there is growing evidence on beneficial effects exerted by breast milk feeding especially in extremely premature infants. Benefits referred to several organs among which CNS, heart, gastrointestinal, and immune system. On this light, it is not matter of speculation to affirm that human milk is still an unknown issue and source of investigation for the future time. The present data show that neuroprotective and neurotrophic proteins such as activin A and S100B as well as HO-1/HSP32 recently demonstrated to empower immune system are not adequately concentrated in milk-formulae milks. Further investigations involving several disciplines (perinatology, biochemistry, and biology engineering) are therefore requested. The aim is to improve knowledge on unconventional biological fluids composition, on laboratory performance and finally on clinical daily practice. These procedures are eagerly awaited bearing in mind that the survival rate in these selected patient populations is dramatically increasing and, in this setting, a maniacal care of all details (technological, medical and nursery, pharmaceutical, feeding, etc.) associated with noninvasive/minimal handling strategies might be the winning strategy.

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