Activin B - Biomaker of ME/CFS

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Reliable serum biomarkers are of immense need for diagnostic purposes of myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS)—a disabling and complex disease for which diagnosis is mainly based on clinical symptoms. The aim of this study was to evaluate a possible diagnostic potential of activin B by directly comparing 134 cases of ME/CFS with 54 healthy controls. Analyses of human activin B level in plasma samples were performed using a validated human activin B ELISA assay.

Keywords: ME/CFS ; human activin B ; visual analogue scale

1. Introduction

Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is a complicated, chronic disease mainly characterized by severe fatigue with many clinical symptoms related to autonomic nervous system imbalance, cognitive impairment, immune and endocrine dysfunction [1]. The prevalence of ME/CFS worldwide varies from 0.1% up to 2.2% depending on the applied diagnostic criteria [2][3][4]. However, there are no European-wide estimates of disease burden [5].

The disease affects all ages, races and socioeconomic groups and some studies show that approximately three to four times as many women as men present with symptoms [6]. Despite many years of molecular and clinical research worldwide, there is still no unified definition for this heterogeneous disease. Another aspect of the complexity of ME/CFS is that no objective parameters or diagnostic markers exist to ensure an exact clinical assessment of the patient. However, the most widely used clinical definitions in clinical research are the Fukuda criteria and the International Consensus criteria, both of which demonstrate an inability to separate ME from CFS [7][8]. Genetic predisposition, stress, trauma, exposure to toxins, physical activity and rest ratio, as well as viral infections have been considered as potential etiological factors for ME/CFS [9][10]. The disease is mainly characterized by severe fatigue, post-exertional malaise, un-refreshing sleep, memory loss, difficulty concentrating, sore throat, lymphadenopathy, muscle pain and headaches. The pathomechanisms of ME/CFS are still under investigation, and there are no standardized biological markers or tests for diagnostics; therefore, even the existence of this medical diagnosis has been questioned for a long time [10][11][12]. The pathogenesis of ME/CFS is likely multi-factorial and various microbial and viral infections are possible trigger factors of ME/CFS. Still, a single infectious representative has not yet been confirmed and the role of viral infections in ME/CFS remains obscure as there is no proven correlation between ME/CFS severity and the stage of infectious process yet.

It is extremely important to develop simpler diagnostic tools from routine data to assist health professionals to diagnose ME/CFS and to monitor therapeutic approaches. Examination of the diagnostic potential of serum biomarkers would allow for the stratification of ME/CFS patients and allow patients to both seek appropriate therapy and evaluate its efficacy in an efficient manner.

As regards possible ME/CFS serum biomarkers, activin B has recently been added to the list. Activin B was stated as a possible marker that could distinguish ME/CFS cases and healthy controls, showing that a higher median of activin B level is observed in healthy controls compared to ME/CFS patients [13]. However, more multi-centre studies demonstrating the previously described tendency with large participant cohorts are needed to acknowledge activin B as a sensitive and specific serum biomarker. Moreover, activin B also had a tendency to predict the severity of the symptoms in patients with ME/CFS. This finding was observed using weighted standing time (WST) severity classes and analysing them together with other serum biomarkers, for example activin A or follistatin [13].

Activins, members of the transforming growth factor β (TGF-β) superfamily, were first isolated from porcine ovarian follicular fluid and identified as activating factors for the release of follicle stimulating hormone [14]. Different additional roles have since been identified for these proteins, including broad and complex effects on cell growth and differentiation, regulation of embryogenesis, development of the reproductive system, wound healing, stem cell differentiation and regulation of immune response [15][16]. Activins are disulfide-linked dimeric molecules in structure composed of βA- and

βA-subunits (activin A), βB- and βB-subunits (activin B), or βA and βB-subunits (activin AB) [14]. Activin A has long been known to be a critical regulator of inflammation and immunity, and similar roles are now emerging for activin B, with which it shares 65% sequence homology [17]. These molecules and their binding protein, follistatin, are widely expressed, and their production is increased in many acute and chronic inflammatory conditions. Synthesis and release of the activins are stimulated by inflammatory cytokines, Toll-like receptor ligands, and oxidative stress. So far, activin A has been the most extensively studied TGF-b family member while activin B has received relatively little attention compared with activin A. In many cases activin B shares several of the functions of activin A; however, it may also exert functionally distinct effects from those of activin A [18]. There are data available showing that activin B production is increased in some cell types in response to inflammatory stimuli, most notably hepatic stellate cells, pituitary cells, and microglial cells [19][20]. Activin B regulates cellular migration by inducing actin stress fiber formation. Activins have been found in most tissues including placenta, reproductive organs, bone marrow, and brain [21].

2. Potential of Activin B as a Clinical Biomarker in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS)

Although ME/CFS has been under investigation for more than 30 years, progress on the examination of the diagnostic potential of serum biomarkers has not been rapid. Trusty serum biomarkers for ME/CFS are essential and necessary for this disabling and complex disease. According to the available data in the literature so far, the role of activin B in ME/CFS has been studied only by one group of researchers from The National Centre for Epidemiology and Public Health, Australia where 45 ME/CFS cases and 17 healthy controls were analysed [22]. By comparing ME/CFS patients with healthy controls, the researchers concluded that a statistically significant increase in activin B level is found in those with ME/CFS ($p < 0.0001$). Despite the relatively small sample size analysed, the authors stated that activin B could help to distinguish ME/CFS patients from those without the condition. Furthermore, the same group of scientists a few years later published a study with an increased ME/CFS group of 85 cases and concluded that activin B level showed a statistically significant decrease in those diagnosed with ME/CFS [13]. Based on data available in the literature about activin B in cases of ME/CFS, we tested the diagnostic potential of activin B by directly comparing 134 cases of ME/CFS with 54 healthy controls. In this study there was no statistically significant difference found regarding the level of activin B between ME/CFS cases and healthy controls, thereby limiting the use of activin B in the diagnostics of ME/CFS. The obtained study results show that the concentration of activin B in patients with ME/CFS and healthy controls do not significantly differ between male and female ($p = 0.0578$; $p = 0.5618$, respectively). Previously published data also show no significant difference between healthy male and female serum samples. However, significant differences were found between healthy values and the other groups (women undergoing IVF procedures, men with marked semen abnormalities and other) of the same gender [23]. The results demonstrate that there were no age-related differences of activin B concentration in healthy adult males. Ambiguous data have been published showing that some studies did not show a difference in activin B levels between healthy men in the age group [23] while others showed an increase in activin B levels in older men [24].

Data published so far show a tendency for activin B to decrease with increasing age of a healthy adult female [23]. That may be associated with a decrease in the number of antral follicles in the ovaries with age, as the antral follicles have been found to express the βB subunit of activin B [25][26]. In this study, the results show that there was no relationship of activin B concentration with age in healthy adult females.

The clinical relevance of activin B has not been clear in ME/CFS; however, the correlation between VAS and activin B level was conducted. The concentration of activin B decreases with increasing VAS score ($r = -0.2004$); however no statistical significance was observed ($p = 0.5085$).

Many factors regulate activin B bioactivity but follistatin is considered as the major regulator [27]. As activin B can bind to follistatin it is very important to choose an assay which measures total activin B, not only “free” activin B, which is unbound to binding protein such as follistatin. The assay is appropriate, because it measures total activin B. The strength of the study is the sufficient number of patients analysed. A limitation of the study can be considered the fact that no simultaneous comparative evaluation of activin B′s closely related sister molecule, activin A, which is also a protein associated with inflammation and tissue stress, has been performed.

Although different ME/CFS diagnostic criteria were used (Canadian consensus criteria in the study mentioned below [22] and Fukuda criteria in the study), comparing the clinical symptoms in the activin B positive group to the ones reported in the cross sectional study regarding activin B [22], the respondents apart from post-exertional fatigue present with difficulty concentrating and sleep disturbances in both cohorts, although respondents in the study were more prone to have myalgia, arthralgia and headache (compared to less than 10% in the study mentioned [22]). There were no patients

presenting with new allergies or arrhythmias in the study, compared to approximately 50% of study participants having these symptoms in the other cohort. As stated above, activin B has the potential role to induce muscle wasting and pain [28]. Nevertheless, one of the core symptoms in the activin B positive group was myalgia, reported by 92%. The fact that 96% reported having it in the activin B negative group may imply that activin B does not influence the clinical presentation of muscle pain in ME/CFS patients. This finding is also substantiated by the fact that the VAS score did not show any statistically significant differences in both groups and even tends to decrease in the activin B positive group. Although any significant differences in clinical presentation of symptoms in both activin B positive and negative groups don't be found, more reports comparing the symptoms in both groups would be needed.

3. Conclusions

Results obtained in our study do not agree with the results of previously published works and do not confirm the clinical applicability of activin B as a biomarker for ME/CFS. Clinically, the respondents with increased activin B levels showed a tendency to have a lower VAS score, although more data is needed to evaluate the correlation.

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