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Therapeutic impact of rHuEPO on abnormal platelet APP, BACE 1, presenilin 1, ADAM 10 and Aβ expressions in chronic kidney disease patients with cognitive dysfunction like Alzheimer's disease: A pilot study

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ABSTRACT

Background: Cognitive dysfunction is reported to be a major cause of morbidity in chronic kidney disease (CKD). The senile plaques (SPs) in the brain are one of the most pathophysiological characteristics of cognitive dysfunction and its major constituent amyloid β (Aβ) released from amyloid precursor protein (APP) by β (BACE1) and γ (presenilin 1) secretases . Platelets contain more than 95% of the circulating APP and implicate as a candidate biomarker for cognitive decline. Recombinant human erythropoietin (rHuEPO) is a standard therapy for anemia in CKD and also acts as a neuroprotective agent. The aim of the study is to determine the impact of rHuEPO therapy on platelet APP processing in CKD with Cognitive Dysfunction.

Methods: A total of 60 subjects comprising of 30 CKD without cognitive dysfunction and 30 CKD with cognitive dysfunction based on neuropsychological assessment. APP, BACE1, Presenilin 1, ADAM 10 (α secretase) and Aβ expressions in platelets were determined by western blotting and lipid peroxidation (LPO) in platelet rich plasma (PRP) was done by spectrophotometrically. The parameters were statistically compared with Alzheimer's disease (AD), Normocytic normochromic anemic and healthy subjects.

Results: Significantly ($p < 0.05$) decreased APP, ADAM 10 while increased BACE1, Presenilin 1, Aβ and LPO were observed in CKD with cognitive dysfunction like AD subjects compared to other groups. The parameters were reassessed in CKD with cognitive dysfunction subjects after rHuEPO (100 IU/ kg, weekly twice, 6 months) therapy. All the parameters were retrieved significantly ($p < 0.05$) along with improved neuropsychological tests scoring after rHuEPO therapy.

Conclusions: This study demonstrated that rHuEPO is an effective neuroprotective agent in the context of CKD associated cognitive dysfunction and proved its clinical usefulness.

1. Introduction

Cognitive impairment is reported to be major cause of morbidity in CKD [1]. The term "cognition" covers aspects of brain function related to various domains such as attention, language, memory, learning, reasoning, decision making, and problem solving [2]. Cognitive impairment and AD are characterized clinically by the presence of both intraneuronal protein clusters composed of paired helical filaments of hyperphosphorylated tau protein (Neurofibrillary tangles, NFT) and extracellular amyloid β (Aβ) protein aggregates (Senile plaques) [3]. Aβ is a 39–43 residue protein with a molecular weight of ˜4 KDa. It is derived by proteolytic cleavage of an integral membrane protein known as amyloid precursor protein (APP) by the action of β- and γ - secretases [3]. APP can undergo proteolysis between positions 671-672 (APP 770 numbering), and anywhere between 710 and 715, to give rise to range of a 39–43 amino acid peptides known as Aβ. Cleavage of the Met-Asp bond at 671–672 generates the N-terminus of Aβ and is catalysed by a protease activity known as β – secretase. Two "β – secretase" proteases have been identified and are known as β site APP cleaving enzymes BACE1 and BACE2 [4]. β – secretase activity is the rate limiting step in

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Aβ peptide production from APP. γ secretase is a multi subunit protease complex, itself an integral membrane protein, that cleaves single pass transmembrane proteins at residues within the transmembrane protein domain. The most well known substrate of γ secretase is APP. Presenilin, an aspartyl protease, is the catalytic subunit of γ secretase. The APP isoforms are expressed in human brain as well as in several non neural tissues and cell lines. Numerous studies support the hypothesis that the presence of APP in peripheral cells, i.e., endothelial and blood cells may contribute to Aβ deposition. Human platelets contain large (> 95%) amounts of circulating APP. Studies implicate platelet APP as a candidate biomarker for dementia and cognitive decline [5–8]. Several studies show blood APP is processed by the same amyloidogenic and non amyloidogenic pathways as utilized in brain and that APP processing in AD patients is altered compared to control subjects and may be a useful biomarker for the diagnosis of AD [6,9–12]. Zainaghi et al. [6] also shows alteration in platelet APP fragments was correlated with membrane and fluidity and cognitive decline. Full length, and processed APP is present in human platelets, which have been shown to contain BACE1 [9] and release Aβ. In the APP proteolytic cleavage, among the 3 secretases, the α secretase (ADAM10) activity prevents the formation of Aβ [13].

Oxidative stress is a state of imbalance between free radicals production and its degradation by antioxidant systems with increased accumulation of the radicals. Oxidative stress has been implicated to play a crucial role in the pathogenesis of neurodegenerative disorders, cancer, and ischemia. Oxidative stress is prevalent in CKD patients and is considered to be an important pathogenic mechanism. CKD patients show an imbalance between excess generation of reactive oxygen species reflected by increased serum indices of lipid peroxidation (LPO) and decreased antioxidant levels [14,15]. Study suggests that the neurotoxic properties of Aβ are mediated by oxidative stress. Oxidative stress is thought to be a key factor in the pathogenesis of AD and mild cognitive impairment [16]. Studies also shown oxidative stress and Aβ production are proportionally linked to each other because Aβ induces oxidative stress in vivo and in vitro [17] and oxidative stress increases the production of Aβ by proteolytic cleavage of APP [18].

Anemia is a common feature of CKD associated with poor outcomes and is typically normocytic, normochromic, and hypoproliferative [19]. The major factor is a relative deficiency of erythropoietin (EPO) synthesis by the failing kidneys [20]. rHuEPO is used routinely to treat anemia in CKD and study shows that along with the level of Hb, cognitive function also improves [21,22]. Experimental studies have shown that rHuEPO exerts a remarkable neuroprotection in both cell cultures and in animal models of nervous system disorders [23–25]. Studies also show that EPO acts as an antioxidant directly by exploiting intracellular antioxidant mechanisms, heme oxygenase 1 and Glutathione peroxidase and several clinical reports have shown that EPO therapy could reduce plasma oxidative stress in CKD patients [26,27]. Based on review of literature, till now there is dearth of knowledge on the level of APP processing and Aβ expression in platelets of CKD patients with cognitive dysfunction when compared with CKD without cognitive dysfunction and also the clinical usage and molecular mechanism of rHuEPO in platelets have not been adequately defined. The aim of the study is to determine the impact of rHuEPO therapy on platelet APP, BACE 1, ADAM10, presenilin 1, Aβ and platelet rich plasma LPO level in CKD with Cognitive Dysfunction and the present study also planned to see if rHuEPO therapy can reverse or reduce these abnormalities. From the studies cited above, it would appear that rHuEPO therapy may have an effect on the level of those proteins expression in CKD patients with cognitive dysfunction.

2. Materials and methods

2.1. Chemicals and drug

The chemicals and reagents were purchased from Himedia and Sisco Research Laboratory (SRL), India. Primary antibodies such as Rabbit Anti-APP polyclonal (bs-0112R), Rabbit Anti-BACE1-Ser498 polyclonal (bs-5215R), Rabbit Anti-presenilin1 polyclonal (bs-0024R), Rabbit Anti-ADAM 10 polyclonal (bs-3574R), Rabbit Anti Beta Amyloid (1–42) polyclonal (bs-0107R), Rabbit Anti beta actin polyclonal (bs – 0061R) and Suitable secondary antibody, Goat Anti Rabbit IgG Antibody (H + L), HRP conjugated were purchased from BIOSS, USA, used to detect APP, BACE1, Presenilin 1, ADAM 10, Aβ and beta actin expressions in platelets by western blot technique.

The rHuEPO was purchased from Alniche Life Sciences Pvt. Ltd, India. For the appropriate subjects, the dose of rHuEPO, 100 IU/kg for 6 months, two times per week was selected according to previous studies [28–30].

2.2. Participants

This experiment was carried out as a *small scale case control study* in Department of Nephrology, SRM Medical college Hospital, SRM University, Kattankulathur, Tamilnadu, India and approved by Institutional ethical committee, SRM Medical college Hospital, SRM University (Clearance No. 58/ IEC/ 2010) and Indian council of Medical Research (No. GIA/ 77/ 2014-DHR), New Delhi, India.

2.2.1. Inclusion criteria

CKD Patients were diagnosed and selected for this study by the nephrologists and either the patient or his relatives who had given informed consent for the study. A total of 60 CKD (Stage V, Age 20–50) patients comprising of 30 CKD without cognitive dysfunction and 30 CKD with cognitive dysfunction based on standard neuropsychological assessment tests i.e. Mini Mental scale examination (MMSE), Wechsler memory scale (WMS I) and Tower of London (TOL) were included for this study (Table 1).

2.2.2. Exclusion criteria

Patient previously diagnosed with Uraemic encephalopathy, Type 1 diabetes mellitus, Malnutrition, Thrombocytopenia, Cerebro Vascular Accident (CVA), patient or relatives who refused to give consent to the study, patient who were dangerously ill and who had very poor medical condition were excluded.

2.3. Methods

CKD patients were clinically evaluated by the clinical psychologist. The patients were subjected to a battery of neuropsychological test to assess memory and functional integrity. The standard questionnaire obtained would be translated into regional language and were subjected to validation.

For screening of cognitive status, CKD patients were administered with standardized Mini Mental State Examination (MMSE) according to the method of Cockrell and Folstein, (1988) [31]. MMSE is a test of global cognitive function with components for concentration, orientation, language, praxis and memory. After conducting MMSE, Wechsler memory scale I (WMS I) was administered to measure different memory functions according to the method of Wechsler [32]. WMS I is made up of seven subtests such as spatial addition, symbol span, design memory, general cognitive screener, logical memory, verbal paired associates and visual reproduction. Person's performance were reported as five index scores such as auditory memory, visual memory, visual working memory, immediate memory and delayed memory.

2.3.1. Tower of London task

Tower of London (TOL) test was administered to the CKD group according to the method of Riccio et al. [33]. The Tower of London test is a well known test used in applied clinical neuropsychology for the assessment of executive function. The TOL Task apparatus consists of two identical wooden boards (30 × 7 × 10 cm) and two sets of three