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Proton NMR based serum metabolic profile correlates with the neurological recovery in treated acute spinal cord injury (ASCI) subjects: A pilot study

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ABSTRACT

Background: Acute Spinal Cord Injury (ASCI) is still having substantial morbidity and mortality despite of advanced therapeutics. Major obstacles are paucity of monitoring tools or biomarkers for severity determination, recovery and prognostication. A prospective case control pilot study with serum ^{1}H NMR spectroscopic metabolic profiling was carried out to evaluate metabolites perturbations and its relationship with recovery and to see role of stem cells in facilitating neurological recovery.

Methodology: Twenty subjects with ASCI were classified on the basis of therapeutic modality into surgical fixation alone (Group-1, n = 10), stem cell adjuvant (Group-2, n = 10) and healthy controls (Group-0, n = 10). Serum samples were collected at admission (baseline) and after six months (follow-up). NMR data of serum sample were quantified and subjected to Wilcoxon and ANOVA tests. Further validation was performed using supervised OSC-PCA and OPLS-DA by incorporating substantial control samples.

Result: Twenty-eight metabolites were identified; well resolved resonances of fifteen metabolites were quantified wherein seven were statistically significant. Predominantly amino acids and ketone bodies played vital role in the differentiation of groups.

Conclusions: Serum NMR spectroscopy reveals certain metabolites perturbations having clear correlation with pattern of recovery in treated ASCI subject. Stem cells treatment group had comparatively effective recovery.

1. Introduction

Spinal cord injury (SCI) is a devastating, severely debilitating traumatic disorder leading to complete or partial disability, affecting the physical and psycho-social wellbeing of the patient. According to WHO, the world wide incidence of SCI is estimated to be between 40 and 80 per million populations per year [1–6]. Despite of best efforts, little has been achieved in terms of neurological recovery. Major successes have been achieved to rehabilitate the subjects to the wheel chair status, by not only modifications in the wheelchair dimensions but also by making the home and society paraplegic friendly. A surgical procedure to decompress the spinal cord and stabilize the fracture segment plays an important role in preventing any further damage. Recent advances in terms of functional electrical stimulation [7], retraining neural circuits to restore body functions and use of adaptive devices for communication are being studied. Ongoing spinal research is vast, in depth, rapidly expanding field involving multidisciplinary studies and working on every dimension possible. Spectacular key concepts are

Abbreviations: ANOVA, Analysis of variance; ASCI, Acute Spinal Cord Injury; BCAAs, Branched-Chain Amino Acids; BDNF, Brain-Derived Neurotrophic Factor; CPMG, Carr-Purcell-Meiboom-Gill; GABA, Gamma-Amino Butyric Acid; HGF, Hepatocyte Growth Factor; HSCs, Hematopoietic Stem Cells; MNC, Mono Nuclear Cell; MSCs or BMSCs, Mesenchymal Stem Cells/Mesenchymal Stromal Cells; NGF, Nerve Growth Factor; NMR, Nuclear Magnetic Resonance; OPLS-DA, Orthogonal Partial Least Square Discriminant Analysis; OSC-PCA, Orthogonal Signal Correction-Principal Component Analysis; SDH, Succinic Dehydrogenase; TSP, Trimethylsilylpropanoic acid; VEGF, Vascular Endothelial Growth Factor

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considered for future directions. Neuroprotection by drugs like steroid, antibiotics, erythropoietin and therapeutic hypothermia; axonal regeneration by anti-inflammatory drugs like Ibuprofen, Indomethacin and rolipram and monoclonal antibodies, anti-MAG (myelin associated glycoproteins) is also under study [8,9] Furthermore, experiments with variety of stem cell such as human oligodendrocyte progenitor cells, schwann cells, bone marrow stromal cells and nasal olfactory ensheathing cells etc. are being under extensive study [10].

In any biological organism there are various organ systems, the functions of which are based on a large number of physiochemical and biochemical reactions [11,12]. These reactions require participation of various small molecular species and a comprehensive study of these molecules is known as metabolomics. A metabolome is defined as a group or collection of low molecular weight compounds with molecular weight < 1 kDa (Kilodalton). The diseases or traumatic events are defined by certain set of patho-physiological changes culminating into a milieu of various intermediate reactionary components or metabolites [13,14]. Earlier plasma ^{1}H-nuclear magnetic resonance (NMR) metabolomics study on rats with SCI has identified certain metabolites belonging to fatty acids and amino acids groups, which clearly differentiated between rats with severe SCI from those of healthy controls [15,16]. This study could be extrapolated to human subjects also. Determining the severity of injury by functional tests and clinical examination alone may not immediately give an accurate assessment of ongoing processes, and especially with respect to predicting future improvement or otherwise [17,18].

In ASCI primary injury occurs at the time of initial mechanical impact causing severe trauma to the cord in the form of contusion, laceration, abrasion, compression, stretch and haemorrhage. It is irreversible and incurable [19]. Secondary injury is a series of processes which occurs in tissues subsequently after primary injury, leading to further progression of inflammation, edema, ischemia, and micro vascular haemorrhage, along with onset of specific pathogenic processes like excitotoxicity due to presence of excess glutamate. Glutamate causes increase in the number of free radicals, ionic dysregulation and immune mediated damages. This phase aggravates the vicious cycle of cell injury and necrosis [20]. It is important to understand the pathophysiology of secondary injury, because it creates a window of therapeutic opportunities for planning interventions. Some studies suggest that surgery (internal fixation), over conservative treatment has a definite role in providing stability and minimizing neurological problems in acute spinal cord injuries.

Stem cells play a versatile role in an organism by maintaining the cellular stability, and indirectly maintaining different biochemical requirements of the body. Several studies have proved that stem cells play different roles including metabolite maintenance during an injury [21,22]. Among all the known sources of stem cells we have chosen bone marrow (BM) cells because of their ease of availability for autologous infusion and do not involve any ethical issues. The BM stem cell transplantation was safe for subjects in short and long-term assessments [23–25].

Curative management of SCI is still a distant dream but innovations of technologies and scientific devices have enabled patients to lead an active, productive and almost near normal life. In quest of new therapeutic modalities and its effect for prognostication and assessment of recovery status by NMR spectroscopy, therefore this pilot study was designed. This is a prospective case-control study where serum samples were analyzed for metabolic alterations and compared with those of healthy controls, to evaluate the status of recovery of subjects suffering from acute spinal cord injury. In addition, the use of stem cells as an adjuvant to conventional treatment has been performed to determine their role in neurological recovery based on metabolomic. The objective of this study was to correlate ^{1}H NMR based serum metabolic profile with the neurological recovery in acute spinal cord injury (ASCI) subjects.

2. Materials and methods

2.1. Case definition

This study was conducted in the Spinal Cord Injury Unit, Department of Orthopaedic Surgery, King George's Medical University (KGMU), in collaboration with the Centre of Biomedical Research, formerly Centre of Biomedical Magnetic Resonance (CBMR), SGPGIMS campus, Lucknow, India. The metabolomics study was ethically approved by the Institutional Ethics Committee (IEC 60th ECM II-B/P14) and stem cell ethics committee (02/ISCES-12) of King George's Medical University. Before enrollment into the study, subjects were explained the study protocol in their local language and their informed consent was obtained. The subjects in the study were recruited as per the inclusion and exclusion criteria.

2.1.1. Inclusion criteria

1. Thoracolumbar injuries with TLISS score ≥4 (Thoraco-lumbar Injury Severity Scale and Score). These are unstable injuries requiring posterior instrumentation by pedicle screw - rod system.
2. ASCI subjects with complete lesion i.e. AIS- A grade (AIS-American Spinal Injury Association Impairment Scale) [26].
3. Age - 18 to 65 years for either gender
4. Duration of injury < 6 weeks
5. Subjects giving informed consent

2.1.2. Exclusion criteria

1. Associated major injuries such as thoraco-abdominal and/or head injury
2. Subjects who did not give informed consent

2.2. Sample size

The sample size of 88 was determined by using method suggested by *Daniel* (1999) by using following formula [27].

$$N = 4Z^2P\,(1 - P)/d^2$$

N = Sample size, P = Prevalence, α = Error, d = Degree of freedom.

$Z_{\alpha/2}$ = Differentiation coefficient (1.96 or 2) and P = 6%.

This being a pilot study, only 10% of parent study sample size was taken for this particular study [28–31].

2.3. Study groups

A total of 30 subjects were enrolled in the study, further twenty ASCI subjects were divided into 2 groups. Group 1 (n = 10) is defined by those ASCI subjects managed by conventional method - Fixation alone (posterior instrumentation with pedicle screw-rod system). Group 2 (n = 10) was ASCI subjects managed by conventional method with augmentation - Fixation with stem cells (Posterior instrumentation with autologous bone marrow derived mononuclear cells rich in stem cells infiltrated at the site of injury). ASCI subjects showing recovery in both the groups were subdivided into 1a and 2a (n = 4 and 6 respectively) for the desired analysis. Ten healthy subjects among the attendants of the ASCI subjects, having no known pathology (age, sex matched), who gave their consent to participate in the study, were enrolled as controls (Group 0). Finally data obtained of these 30 subjects were analyzed by univariate analysis. Serum samples of ASCI subjects as well as those of controls were collected at the time of admission (baseline) and after six months (follow-up). These spinal injury subjects were provided the best surgical, medical as well as rehabilitation facilities.