A Proton NMR study of Serum and Urine in pre and post nutritional intervention in Buerger’s Disease
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Introduction:
Buerger’s disease is a non-atherosclerotic vascular disease, also known as thromboangiitis obliterans (TAO). It is characterized by the absence or minimal presence of atheromas, segmental vascular inflammation, vaso-occlusive phenomenon, and involvement of small- and medium-sized arteries and veins of the upper and lower extremities. This clinical condition primarily afflicts poor population those who smoke heavily. The initial symptoms involve pain during walking followed by pain at rest, ulceration and gangrene of the toe and foot. The mechanism underlying Buerger’s disease is still largely unknown ranging from hypo-vitaminosis to homocysteine metabolism to other genetic deficiencies. Till today the management of the disease is surgical in the form of Lumbar sympathectomy with a high failure rate. A nutritional treatment modality oral administration of 15g/day of L arginine (to increase NO production and decrease vasospasm), folic acid (5 mg), vitamin B6 (100 mg) and vitamin B12 (2000 mcg) has been attempted in the present study and the metabolic changes in the urine and serum of patients of Buerger’s disease were identified pre and post treatment by ¹H NMR as well as nitric oxide (NO) estimation with ELISA.

Material and methods: All patients (males) suffering from Buerger’s disease (median age 30) were included in the study. The current study is a prospective observational study of in-vitro ¹H NMR analysis of serum and urine samples from healthy controls (n=26) and patients of Buerger’s disease (n=16) and its correlation with nitric oxide level determination of serum of patients of Buerger’s disease. Patients with other autoimmune disease, hyper-coagulable states and diabetes mellitus and with evidence of a proximal source of emboli by echocardiography/arteriography were excluded from the study. Urine and serum sample for NMR analysis were collected in cryogenic vial and stored at – 80°C till NMR analysis. All NMR experiments were carried out at 25°C, unless otherwise stated. ¹H NMR experiments were performed on Bruker BioSpin Avance II 800 MHz with cryoprobe FT-NMR spectrometer equipped with 5mm ¹H/13C/15N cryoprobe equipped with Z-shielded gradient. One-dimensional ¹H NMR experiments were performed with a single-pulse sequence and suppressing the residual water signal by presaturation with 32K time-domain data points, a spectral width (SW) of 5952 Hz and 128 scans. The flip angle of radio-frequency pulse was 45° with the total relaxation delay of 7.04 s. Free-induction decays were multiplied by an exponential function with a line-broadening of 0.30 Hz, prior to Fourier transformation. The data, thus obtained, was subjected to unsupervised multivariate Principal Component Analysis. The concentration of NO was determined spectrophotometrically using nitric oxide colorometric assay kit.

Results:

![Typical ¹H CPMG NMR spectra of serum samples of control and diseased group are shown in Figure 1(a). Major differences were observed in concentration of lipoproteins (fatty acids), glutamate, serine, tyrosine, branched chain amino acids, lactate, alanine, and phenylalanine (red dots indicate significance). Its corresponding PC-1, PC-2 versus PC-3 3D score plot is shown in Figure 1(b), which clearly distinguishes three sets of groups, one corresponding to control and other two corresponding to diseased and post-treatment states. Figure 1(c) shows differences among the patients with higher and lower nitric oxide levels.](image)

Discussion: Buerger’s disease can be mimicked by a wide variety of other diseases that cause diminished blood flow to the extremities. These other disorders must be ruled out against an evasive evaluation, because their treatments differ substantially from that of Buerger’s disease. For Buerger’s disease, there is no treatment known to be effective. Therefore, metabolic biomarkers were identified with the help of ¹H NMR spectroscopy and were correlated with serum nitric oxide levels for the determination of extent of severity of disease. Also, post-nutritional L-arginine (15 g/day) was administered as an approach to increase vasodilatory NO production via endothelial NO synthase (eNOS). Within 8 weeks on therapy, a substantial clinical improvement with reduced pain, gangrenous ulcers in the foot and leg along with normalized arterial pulses of the patients was observed. NO levels correlate linearly with the vascular tone and are proven to be actively involved in reducing oxidative stress (2). The higher NO level (10±1.2) indicated the mild stage of disease while the lower levels (5±2.3) indicated the severity of disease. Whereas, urine profiling indicated ambiguity in classification, the ¹H NMR based study of serum showed good correlation and separation among mild and severe diseased patients serum and among controls along with treated patients. This study indicated the alterations in fatty acid metabolism of patients suffering with Buerger’s disease along with the significant differences in glutamate, tyrosine, serine and phenylalanine concentrations. The variation in serine concentration indicates metabolic perturbations in homocysteine pathway as well.

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