Abstract on

"NMR based Metabolomics of Synovial Fluid from patient with Reactive Arthritis (ReA) for Identifying abnormal metabolic status"

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By

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Abstract

Reactive arthritis (ReA) is a member of the spondyloarthropathy family. The Reactive arthritis is an infectious disease which affects mostly to young adults. Broadly, reactive arthritis is arthritis induced by infections pathogens in which the pathogens cannot be recovered from the joints. Recently, non-infectious components of the infective organism have been demonstrated in the synovium, and synovial fluid and the presence of microbial components in the joints of patients with ReA have demonstrated the need for improved diagnostic criteria.

The bacterial species which are commonly studied include Chlamydia trachomatis, Salmonella typhimurium or enteritidis, Shigella flexneri and Campylobacter jejuni. In most instances the primary infection affects either the gastrointestinal or the urogenital tract; hence the terms enteroarthritis or uroarthritis. Also, respiratory tract infections by Chlamydia pneumoniae, for example, may play the triggering role in ReA. An important aspect to keep in mind in clinical practice is that the primary triggering infection may pass with very mild symptoms or may even be symptomless, and its severity is not at all related to the severity of the later ReA. A previously healthy but genetically predisposed individual who contracts a suitable triggering infection will develop ReA after some time. This pathogenetic process spans the incubation time of the triggering infection and its clinically overt phase, followed a few days to a couple of weeks later by full-blown ReA. An important factor associated with the susceptibility of an individual to ReA is HLA-B27. It appears that in B27-positive individuals the disease is more severe and the tendency for chronicity greater than in those who are B27 Negative. The clinical picture of reactive arthritis varies from mild arthralgias to severely disabling conditions. This disease, in spite of its name, is not restricted to the joints but affects several different organs and tissues.

The worldwide incidence of ReA is approximately 5 to 14 cases per a 100,000 population depending geographic locations. ReA usually affects young people aged 18–60 years with almost the same frequency among males and females. The incidence of enteroarthritis or uroarthritis per 100,000 adults per year is 30, and that of seronegative arthritis is 40 per 100,000. Rheumatic fever also a type of reactive arthritis, often occurs in developing countries but is rare in developed countries. However, it was previously claimed to be more common in males. Most patients are aged between 20 to 40 years. For over two decades extensive efforts have been devoted by several groups to clarify the mechanisms behind this association. The different HLA-B27 loci have been characterised in minute detail, and the peptides bound by this structure analysed. In 65%-85% of patients, ReA is associated with the class I major histocompatibility antigen HLA-B27. The majority, in some studies up to 90%, of the patients contracting reactive arthritis after infection with Chlamydia, Salmonella, Shigella or Yersinia are HLA-B27 positive.

ReA is a systemic disease which having extra-articular symptoms. The clinical picture is prevailed by peripheral arthritis (acute or subacuteoligoarthritis mainly of the lower limb joints), enthesitis (enthesopaties), pelvic and axial syndromes (spinal involvement with sacroiliitis) and extra-musculoskeletal syndromes. The acute and chronic symptoms can include articular, tendon, mucosal, cutaneous, ocular, and occasionally cardiac manifestations or systemic features (fever, malaise, and weight loss); the latter usually are confined to the acute stage. Symptoms typically start within 1 to 4 weeks of the initial infection.

Intensive research during the last two decades has provided an impressive amount of new information regarding ReA. Yet, the pathogenesis is still not fully understood, and therefore no proper therapy or prophylaxis exists. More studies have been undertaken in biomarker discovery due to the potential for biomarkers to be used in the diagnosis of disease prior to other clinical signs. Biomarkers are biological molecules that can show biological processes and pathological states of individual organisms. These biomarkers can then be utilized to determine if that individual is healthy, diseased or pre-disposed to a disease. In the case of disease diagnosis, biomarker profiles of body fluids may be used for diagnosis before the discernment of other clinical signs of the disease. Biomarkers consist of chemicals from a range of chemical types and include DNA, mRNA, proteins and metabolites, and these biomolecules can be observed in a range of biological fluids. Fluids are generally selected by their ease of accessibility and practicality and can be obtained from serum, plasma, whole blood, urine, saliva, sweat, ascites fluid, cerebrospinal fluid (CSF), synovial fluid, hair or feces. There are two unlike approaches that can be used in biomarker discovery: targeted and non-targeted. Targeted approaches look at specifically known biomolecules; sub-classes of biomolecules such as esters or amines; or the metabolites involved in a certain metabolomic cycle. Non-targeted approaches examine as many molecules as possible at once so that the complete metabolome can be observed and allow for biomarker discovery without prior knowledge of the biological pathway of the disease. In the case of metabolites, searching for biomarkers using a non-targeted approach requires universal methods that have the ability to simultaneously separate and detect metabolites from multiple chemical classes. It can be difficult because of the range of physical and chemical properties of the different metabolites. One technique is, therefore, not suitable to examine all types of metabolites simultaneously.

Metabolomics a new-sprung cousin to genomics and proteomics- is an analytical approach to metabolism that involves a comparative and quantitative analysis of low molecular weight metabolites and their intermediates concentration profiling in affected biological systems (typically urine, blood plasma/serum/ synovial fluid, cell lysates, or tissue extracts). Genomics,

transcriptomics and proteomics analysis further complemented with metabolomics information, which allows offers the potential to understand the entire biological system including health or disease processes operating in that system –so-called systems biology approach. With its ability to discover disease-related biomarkers and underlying biochemical processes, nowadays, metabolomics is utilized virtually in all aspects of biomedical research directed to enhance the understanding of the disease and health processes. The complete paradigm is based on the fact that a pathophysiological condition or therapeutic intervention results in a distinct and characteristic change in the biochemical composition profiles of biofluids and metabolomics aims to identify these changes. The biochemical changes -that correlate with a disease (or disease type/grade) and treatment response- then permit the clinical researchers to raise the diagnosis of disease including, early disease detection, monitoring response to treatment and patient stratification for therapy. The molecular biomarkers validated using wide-range of human populations form the basis for new clinical diagnostic assays.

Nowadays, Proton Nuclear Magnetic Resonance (^{1}H NMR), Gas Chromatography-Mass Spectrometry (GC-MS) and Liquid Chromatography-Mass Spectrometry (LC-MS) are well-established powerful analytical methods for generating metabolomics profiles. For the analysis of complex, biological samples like bio-fluids, these techniques have their advantages and disadvantages. For instance, GC-MS requires derivatization, which lengthens the sample preparation time. In general LC-MS and GC-MS need more time-consuming sample preparation.

NMR offers several clear advantages compared to other analytical and biochemical analysis methods used for metabolomics studies: (a) applicable to a variety of biological and clinical samples, tissue extracts, and even cell lysates, (b) rapid, quantitative, and offers the potential for high-throughput (i.e. analysis of >100 samples/day is attainable), (c) least-destructive (i.e. the prepared sample can be used in many consecutive NMR experiments or the same sample can be analysed by the other-other analytical techniques after the NMR experiments are completed), (d) unbiased (i.e., all protonated metabolites present in a biological mixture are detectable irrespective of their physical properties) (e) Peaks in the NMR spectra can be consistently assigned to the particular metabolite, based on their chemical shifts and multiplet patterns and (f) virtually it requires no sample preparation and furnishes highly reproducible results. These are the main grounds that NMR has become the method of choice for studying metabolic changes associated with distinct human pathologies and also gaining tremendous popularity in pharmaco-metabolomics studies as well. Nowadays, the sensitivity of NMR is also not a major issue; even nanogram detection limits are possible with novel pulse methodology and appropriate instrumentation.

The main objective in Metabolomics is to study all the metabolites within a biological sample in an unbiased manner. The metabolomics studies involve a number of specimens together for the analysis. For metabolomics, well-planned experiment design and execution are required along with univariate and multivariate statistical analysis is needed for pattern recognition and interpretation of metabolomics data. Hence, the NMR spectroscopy coupled with multivariate statistical analysis permits the identification of metabolic disturbances and distinct metabolic pathways associated with the disease, but it also allows the recognition of metabolic signatures which have their potential diagnostic and prognostic implications for clinical management of the disease.

The NMR-based metabolomics approach has already been helpful in identification of metabolic markers for inflammatory rheumatic disease such as Systemic lupus erythematosus (SLE), ankylosing spondylitis (AS) rheumatoid arthritis (RA), and gout disease etc. The identification of disease biomarkers plays a critical role not only in early disease diagnosis or risk prediction but also in classification and disease progression or assessment prognosis and treatment response. Establishment of these biomarkers in routine clinical use has the potential to provide insight into the pathogenesis of disease states and discover diagnostic markers for therapeutic targets. Thus, the metabolic biomarkers have the power to increase the overall survival and quality of patient life in, addition to saving huge expenses for the society. The primary objective of the research presented in this thesis was to evaluate the use of high-resolution NMR spectroscopy together with multivariate analysis based metabolomics for identifying and characterizing potential biomarkers of ReA patient. This thesis consists of original research work in which the applicability of NMR Metabolomics in identifying biomarkers of disease dependent changes has been explored. Metabolomic studies were performed on a broad range of subjects ranging from healthy volunteers to patients with advanced stage of the disease. The research objectives undertaken has been discussed briefly.

Findings of Objective 1: Currently, there are no reliable clinical biomarkers available that can assist early differential diagnosis of reactive arthritis (ReA) from other inflammatory joint diseases such as RA. Metabolic profiling of synovial fluid (SF) obtained from joints affected in ReA- holds great promise in this respect and will further aid monitoring treatment and improving our understanding about disease mechanism. As the first step in this pivot, we describe here the metabolite assignment of ^{1}H and ^{13}C resonances observed in the NMR spectra of SF samples collected from patients of ReA. The metabolite characterization has been accomplished on both normal as well as on ultra-filtered SF samples of eight ReA patients (n=8) using high resolution (800 MHz) ^{1}H and ^{1}H-^{13}C NMR spectroscopy methods such as one-dimensional (1D) ^{1}H CPMG

and two-dimensional (2D) J-resolved ^{1}H NMR and homonuclear ^{1}H-^{1}H TOCSY and heteronuclear ^{1}H-^{13}C HSQC correlation spectra. Compared to normal SF samples, several distinctive ^{1}H NMR signals were identified and assigned to metabolites in the ^{1}H NMR spectra of ultra-filtered SF samples. Overall, we assigned 53 metabolites in normal filtered SF and 64 metabolites in filtered pooled SF sample compared to non-filtered SF samples for which only 48 metabolites (including lipid/membrane metabolites as well) have been identified. The established NMR characterization of SF metabolites will serve to guide future metabolomics studies aiming to identify/evaluate the SF based metabolic biomarkers of diagnostic/prognostic potential or seeking biochemical insights into disease mechanisms in a clinical perspective.

Findings of Objective 2: Reactive arthritis (ReA) is a member of seronegative spondyloarthropathy (SSA) group which involves an acute onset of asymmetrical lower limb joint inflammation following weeks after a genitourinary/gastrointestinal infection. The diagnosis is clinical as it is difficult to culture the microbes from synovial fluid. Arthritis patients with the similar clinical picture, but, lapsed history of immediate preceding infection and did not fulfill the diagnostic criteria of other members of SSA group such as psoriatic arthritis, ankylosing spondylitis, and arthritis associated with inflammatory-bowel-disease, are marked as peripheral undifferentiated spondyloarthropathy (uSpA). ReA/uSpA patients show a strong association with Class-I major-histocompatibility-complex (MHC) allele, HLA-B27; however, the disease mechanism is far from clear. As the clinical picture is largely dominated by Rheumatoid arthritis (RA) like features including enhanced levels of inflammatory markers (such as ESR, CRP, etc.). These overlapping symptoms often confound the clinical diagnosis and represent a clinical dilemma making treatment choice more generalized. Therefore, there is an obliging need to identify biomarkers that can support the diagnosis of ReA/uSpA . In present study, we performed NMR based serum-metabolomics analysis and demonstrated that ReA/uSpA patients are clearly distinguishable from controls and further these patients can also be distinguished from the RA patients based on the metabolic profiles, both with high sensitivity and specificity.

Present study concluded on the basis of area under receiver operating characteristic (AUROC) curve analysis and led to the recognition of four metabolic entities (i.e. valine, leucine, arginine/lysine and phenylalanine) which could differentiate ReA/uSpA from RA.