

IPD Project Details

Project ID: IPD4797

Project Title: Quantitative Proteomics analysis of Plasmodium vivax induced alterations in human serum during the acute and convalescent phases of infection

Description: Malaria is by far the world's most significant tropical infectious disease and over the last a few decades large-scale malaria epidemics have happened in almost all continents. Plasmodium falciparum and Plasmodium vivax account for over 90% of the total malaria cases worldwide. The estimated number of annual clinical cases of vivax malaria is even higher than that of falciparum malaria, but yet the morbidity associated with this infection and its spectrum of disease is largely neglected. Identification of serum/plasma proteins, which exhibit altered abundance at the onset and during the acute phase of any infection, could be informative to understand the pathobiology of different infectious diseases and host responses against the invading pathogens. To this end, in recent years, quite a few research groups including us have investigated alterations in serum/plasma proteome in severe and non-severe falciparum malaria (and also vivax malaria) to study malaria pathogenesis. In all these studies, serum/plasma proteome of the malaria patients have been analyzed during the febrile stages of the infection, either at the onset of the disease or at the fastigium stage. However, temporal profiling of serum/plasma proteome during acute and remission stages in malaria, which can provide snapshots of the transient and enduring alterations in serum proteome during the febrile, defervescence and convalescent stages has not been reported hitherto. Here, we report, for the first time, serum proteomic alterations in a longitudinal cohort of P. vivax infected patients to elucidate host responses when fever is established (temperature of the body reaches above higher normal level), during the stage when the temperature comes down to normal, and also during the gradual recovery of health after the illness. The three stages discussed in our study have been categorically chosen depending upon the clinical course of uncomplicated vivax malaria. Analysis of the early febrile stage represents host proteome profile immediately after onset of the infection, without any effect of anti-malarial drugs. The second, defervescence stage, reflects any immediate change in blood proteome at early recovery phase, while the convalescent stage indicates a phase after administration of 14 days radical cure treatment with primaquine and a complete recovery, when none of the patients displayed any apparent symptoms of malaria. We have also performed an extensive quantitative proteomics analysis to compare the serum proteome profiles of vivax malaria patients with low and moderately-high parasitemia with healthy community controls. Isobaric tags for relative and absolute quantitation (iTRAQ) and 2-D

fluorescence difference gel electrophoresis (2D-DIGE)-based quantitative proteomics approaches were used in the discovery-phase of the study, and some selected differentially abundant serum proteins were validated further by using ELISA. Interestingly, some of the serum proteins like Serum amyloid A, Apolipoprotein A1, C-reactive protein, Titin and Haptoglobin, were found to be sequentially altered with respect to increased parasite counts, while many of the quantified candidates such as Hemopexin, Vitronectin, Clusterin and Apolipoprotein E exhibited nearly equal levels of differential serum abundance in different parasitemic malaria patients. Analysis of a longitudinal cohort of malaria patients indicated reversible alterations in serum levels of some proteins such as Haptoglobin, Apolipoprotein E, Apolipoprotein A1, Carbonic anhydrase 1, and Hemoglobin subunit alpha upon treatment; however, the levels of a few other proteins did not return to the baseline even during the convalescent phase of the infection. Identification of the differentially abundant serum proteins and associated physiological pathways in vivax malaria along with phase-specific protein profiles during the acute and convalescent phases of the infection can effectively enhance our understanding of *P. vivax* disease biology and host immune responses.

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Sample Preparation: Twenty-three low parasitemic (LPVM, parasite count < 200/μL of blood) and 40 moderately-high parasitemic (MPVM, parasite count > 2000/μL of blood) non-severe vivax malaria patients classified according to the WHO guidelines, along with 40 age and gender-matched healthy controls (HC) were enrolled for the study. In addition, In order to perform a longitudinal study, serum samples were collected during early febrile phase immediately after diagnosis, before administration of any antimalarials (FEB; day 0), defervescence (DEF; day 2) and convalescence (CON; day 15 ± 3) stages. Blood samples (5.0 mL) were collected from the antecubital vein of the subjects using serum separation tubes (BD Vacutainer®; BD Biosciences). Immediately after blood collection the tubes were kept in ice for 30 minutes for clotting. After clotting, the samples were centrifuged at 2500 rpm at 200C for 10 minutes and serum was collected carefully from the upper surface. Collected serum was divided into multiple aliquots and stored at - 800C until time of analysis to prevent protein degradation. Prior processing of serum samples, the top two high abundant proteins, albumin and IgG were removed. Protein extraction from depleted serum samples was performed using TCA/acetone precipitation method. Protein samples (in rehydration solution were exchanged to TEAB buffer using Amicon Ultra 0.5 mL centrifugal 3 kDa filters (Millipore, Watford, UK). After buffer exchange, quantification of protein content in each sample was performed using QuickStart Bradford reagent (BioRad, USA). Quantitative proteomics analysis was performed on HC, LPVM and MPVM cohorts using the pooled samples (each pool containing 20 samples). Sample labeling strategy for differential proteomic analysis was; HC-114, LPVM-115 and MPVM-116. Comparative serum

proteome analysis of vivax malaria patients at three different time points against healthy controls was also performed using iTRAQ. 10 selected serum samples from each of the study cohorts (T1, T2, T3 and HC) were split into three pools- Set 1(n = 4); Set 2 (n = 3) and Set 3 (n = 3). Apart from these biological replicates, a pool containing all 10 samples (T1, T2, T3 and HC) was also analyzed. HC samples were labeled with the 114 iTRAQ reagent, while the three different time-point samples (FEB, DEF and CON) were labeled with 115, 116 and 117 labels, respectively.

Peptide Separation: Prior to the iTRAQ labeling, in-solution digestion was performed (75?g proteins from each sample) following the manufacturer's instructions. After in-solution digestion, iTRAQ labeling of the peptides was performed following the manufacturer's instructions (AB Sciex, USA). OFFGEL fractionation of the labeled peptides was performed using a 3100 OFFGEL fractionator (Agilent Technologies, Santa Clara, CA) with high resolution (pH 4-7, 24 cm) IPG strips. Agilent 6550 iFunnel Q-TOF LC MS/MS instrument (Agilent Technologies, USA) equipped with a Chip-Cube controlled by the Mass hunter Acquisition software in a positive ion mode was applied for quantitative proteomic analysis. The range of the MS spectra was kept between 300-2000 and for MS/MS 50-3000. The instrument was operated using AutoMS/MS, in a data operated manner, maximum 4 precursor ion peaks were selected having intensity over 1000 for each cycle. The gas pressure was maintained at 2×10^{-2} bars in the collision cell for MS/MS analysis. Mass tolerance of 20 ppm and fragment mass tolerance of 50 ppm was specified.

Protein Characterization: Protein identification and quantification of the iTRAQ reporter ion intensities were performed using Spectrum Mill identification software (Agilent Technologies, USA). The Paragon algorithm was used as default method for search with trypsin as a digesting agent and IAA for cysteine and iTRAQ (N-term K) were selected for the fixed modification and oxidized methionine as the variable modification. Peptide mass spectra obtained were searched against the UniProt database. p-values obtained from a paired t-test were used to evaluate significance of differences in the protein abundances between control and malaria study cohorts. P-values (adjusted) < 0.05 were considered to be statistically significant.

Experiment Type: Shotgun proteomics

Species: Homo sapiens-9606

Tissue: Blood serum (bto:0000133)

Cell Type:

Disease: Plasmodium vivax malaria (doid:12978)

Instrument Details: 6220 Time-of-Flight LC/MS (MS:1000675)

Protein Modifications: iTRAQ4plex-116 reporter+balance reagent acylated residue

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