

## **Supplementary Material**

After getting informed consent from the patients, blood samples were collected according to the study protocol. Before processing, thin blood smear was prepared on clean glass slides for microscopic analysis. An aliquot was used for routine laboratory hematology, second for separation of plasma and erythrocyte lysates, third for separation of plasma and buffy coat and fourth for separation of lymphocyte. Blood samples from the control subjects were also processed and analyzed using the same protocol. The details of the chemicals along with experimental procedures are described in Annexure I – Annexure III.

### **Annexure I**

#### **REAGENTS AND CHEMICALS USED IN THE CURRENT STUDY**

Ethylenediaminetetraacetic acid (CAS no. 60-00-4), Dimethyl formamide (CAS no. 68-12-2), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (CAS no. 7365-45-9), 2,7-dichlorofluorescein diacetate (CAS no. 2044-85-1), Histopaque®-1077 (CAS no: 737-31-5), Microscopic slides (cat no: S8902), Distrene plasticizer xylene (Cat no: 31,761-6), Paraformaldehyde (CAS no: 30525-89-4), Triton X-100 (CAS no: 9002-93-1), Bovine serum albumin (CAS no: 9048-46-8), Sodium chloride (CAS no: 7647-14-5) were purchased from Sigma Aldrich (St. Louis, MO, USA). Phosphate buffered saline (cat no: 17-517Q) was obtained from Lonza (Walkersville, MD, USA). Total reactive oxygen species detection kit for microscopy (cat no: ENZ-51011) and human oxidized low density lipoprotein Enzyme-linked immunosorbent assay kit (cat no: 10-1143-01) were purchased from Mercodia Inc (Winston Salem, NC, USA) and Enzo Life Sciences Inc. (Farmingdale, NY, USA), respectively. Superoxide dismutase detection kit (cat no: CSOD100-2) was from Cell Technology Inc. (Mountain View, CA, USA). Primary antibodies rabbit polyclonal anti- $\gamma$ -H2AX phospho S139 (cat no: ab2893), anti-Mre-11 (cat no: ab33125), anti-DNA Ligase IV (cat no: ab80514), anti-Ku70 (cat no: ab83501) and anti-Ku80 (cat no: ab87860) were purchased from Abcam Inc. (Cambridge, MA, USA). Secondary antibodies goat anti-rabbit IgG alexa fluor® 488 (green; cat no: A-11008), alexa fluor® 594 (red; cat no: A-11012), DAPI (blue; cat no: D1306) and ProLong® Gold antifade reagent (cat no: P36934) were purchased from Life Technologies (Grand Island, NY, USA).

## Annexure II

### **ANALYSIS OF OXIDATIVE STRESS AND ANTIOXIDANT DEFENSE**

#### **A. Flow cytometric measurement of ROS generation in blood leukocytes**

Generation of reactive oxygen species (ROS) was measured in blood leukocytes (granulocytes, monocytes, and lymphocytes) by flow cytometry using 2',7'-dichlorofluorescein diacetate (DCF-DA) following the procedure of Rothe and Valet (1990) [27] with slight modification [28]. In brief, an aliquot of blood was centrifuged at 200×g at 4<sup>0</sup>C for 10 min and the buffy coat containing leukocytes and supernatant plasma were collected separately. An aliquot of 200 µl buffy coat was mixed with 300 µl of freshly collected plasma. Then 10 µl of the mixture was diluted with 1ml of HBSS containing 0.15M NaCl and 5mM HEPES, pH7.35. Thereafter, 20 µl of 0.5mM DCF-DA solution in dimethyl formamide was added to the cell suspension and incubated at 37<sup>0</sup>C for 30 min in darkness. After washing in ice-cold PBS, 10,000 events were acquired immediately in flow cytometer (FACS Calibur with sorter, Becton Dickinson, San Jose, CA, USA) using Cell Quest software (BD, USA). The neutrophils, monocytes and lymphocytes were gated on the basis of their characteristic forward and side scatters on dot plot. Generation of ROS by the leukocytes resulted in green fluorescence that was recorded in fluorescence channel-1 and was expressed as mean fluorescence intensity (MFI) in arbitrary unit for each leukocyte subset.

#### **B. Microscopic evaluation of ROS generation in blood leukocytes**

This assay was conducted for visual detection of intracellular ROS production in blood leukocytes by fluorescence microscopy. ROS was detected using the commercially available ROS detection kit according to the manufacturer's instructions with slight modification. In brief, 400 µl mixture of buffy coat containing leukocytes and supernatant plasma (as described before) were used for this assay. The mixture was centrifuged at 400×g at 4<sup>0</sup>C for 5 min. The cell pellet was collected and re-suspended in 200 µl of the ROS detection solution and incubated at 37<sup>0</sup>C for 1 hour in darkness with periodic shaking. Then the cell suspension was centrifuged at 400×g for 5 minutes. After supernatant was removed, the cell pellet was re-suspended the cells in 100 µl of 1× Wash Buffer. A 20 µl aliquot of the cell suspension was applied onto a microscope slide. The cells were immediately overlaid with a cover slip and analyzed via inverted fluorescence microscope (Olympus IX71, USA) equipped with ProgRes C3 camera (Jenoptik;

Laser Optik System GmbH, Germany). Intracellular ROS was visualized in the cytoplasm of leukocytes as green fluorescence.

### **C. Spectrophotometric measurement of SOD in erythrocytes**

The activity of the antioxidant enzyme superoxide dismutase (SOD) was assayed in blood erythrocyte lysate spectrophotometrically using commercially available kit following the instruction of the manufacturer. The absorbance (OD) was measured at 450 nm using SpectraMax® M3 multi-mode microplate reader (Molecular Devices, California, USA). SOD activity was calculated using SoftMax Pro software (Molecular Devices, California, USA) and expressed as units per milliliters (U/mL).

### **D. Measurement of oxidized LDL in plasma**

The concentration of oxLDL in plasma was measured by ELISA using a commercially available kit following the manufacturer's protocol. Each sample was assayed in duplicate. The lowest detection limit of the kit was 1.0 mU/L.

## **Annexure III**

### **ISOLATION OF BLOOD LYMPHOCYTES AND IMMUNOFLUORESCENCE MICROSCOPY**

An aliquot of EDTA-anticoagulated whole blood was centrifuged for 10 min at 400×g at 4<sup>0</sup>C in Histopaque density gradient following the instruction of the manufacturer. The lymphocytes separated in buffy coat were collected in microfuge tubes. Twenty micro liter cell suspension was used for smear preparation on clean glass slides for immunocytochemistry. Air dried smears were fixed in 4% paraformaldehyde for 15 min, washed twice in 0.1 M PBS, pH 7.2, and subsequently incubated for 5 min in 0.1 M PBS containing 0.2% Triton X-100 to permeabilize cell membranes followed by two washing steps with 0.1 M PBS. After washing in PBS, nonspecific binding was blocked by incubating in 3% BSA at room temperature for 1.5 hrs. The slides were incubated separately in darkness with rabbit polyclonal primary antibodies (anti- $\gamma$ -H2AX phospho S139, dilution: 1:1000; anti-Mre-11, dilution: 1:200; anti-DNA Ligase IV, dilution: 1:500; anti-Ku70, dilution: 1:100 and anti-Ku80, dilution: 1:100 in 1% BSA) at room temperature for 2 hrs. The secondary antibodies goat anti-rabbit IgG Alexa Fluor® 488 (green, dilution: 1:500) for  $\gamma$ -H2AX and DNA Ligase IV and Alexa Fluor® 594 (red, dilution: 1:300) for

Mre-11, Ku70 and Ku80 were used for 1 hr. DAPI (blue) was used to stain the cell nuclei at a concentration of 1.43 $\mu$ M. To suppress photobleaching and preserve the signals of fluorescently labeled target we used ProLong® Gold antifade reagents molecules to mount the slides with cover slips. Slides were analyzed under an inverted fluorescence microscope (Olympus IX71, USA) equipped with ProgRes C3 camera (Jenoptik; Laser Optik System GmbH, Germany). To prevent bias in selection of cells that display foci ( $\gamma$ -H2AX and Mre-11), all non-overlapping cells were counted in the field of vision. On the other hand, 200 non overlapping cells were counted for the presence of positive nuclei (DNA Ligase IV, Ku70 and Ku80) and represented as percent positive cells for each slide. To control for non-specific binding of secondary antibodies, negative control experiments were performed, which were handled similarly, with the exception that the cells were incubated in PBS/BSA instead of primary antibody. Image Pro Plus (Media Cybernetics, Inc., Rockville, MD, USA) was used for analysis of slides.