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FULL LENGTH ARTICLE

Anti-endothelial cell antibody rich sera from rheumatic heart disease patients induces proinflammatory phenotype and methylation alteration in endothelial cells

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Abstract Rheumatic heart disease (RHD) is a major cause of cardiovascular morbidity and mortality in developing nations like India. RHD commonly affects the mitral valve which is lined by a single layer of endothelial cells (ECs). The role of ECs in mitral valve damage during RHD is not well elucidated. In here, anti-endothelial cell antibody from RHD patients has been used to stimulate the ECs (HUVECs and HMVECs). ECs proinflammatory phenotype with increased expression of TNFα, IL-6, IL-8, IFNγ, IL-1β, ICAM1, VCAM1, E-selectin, laminin B, and vimentin was documented in both ECs. The promoter hypomethylation of various key inflammatory cytokines (TNFα, IL-6, and IL-8), integrin (ICAM1) associated with leukocyte transendothelial migration, and extracellular matrix genes (vimentin, and laminin) were also observed. Further, the *in-vitro* data was in accordance with *ex-vivo* observations which correlated significantly with the etiological factors such as smoking, socioeconomic status, and housing. Thus, the

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study sheds light on the role of ECs in RHD which is a step forward in the elucidation of disease pathogenesis.

Introduction

Rheumatic heart disease (RHD) is one of the most common valvular disease affecting scores of patients in developing countries with few sporadic cases in developed countries. In 2015, RHD accounted for 33 million cases and 319,000 deaths worldwide out of which 13 million cases and 119,000 deaths were reported from India.[1] Although, various molecular and pathogenic factors have been attributed including genetic predisposition,[2-4] molecular mimicry with heart and endothelial proteins and GAS (Group A streptococcus or *Streptococcus pyogenes*) antigens,[5] yet the exact mechanism of RHD pathogenesis remains undeciphered.

In RHD, the mitral valve is predominantly affected. It is lined by a single layer of endothelial cells (ECs) which is believed to have a pertinent role in RHD development.[6] Additionally, RHD is a multifactorial disease which involves the environment, host, and pathogen. These three factors determine the disease causation and the degree of severity.[7] Environment and the pathogen are believed to be altering the host epigenetic signatures to establish the disease as evident in various autoimmune and infectious diseases.[8] The nonheritable epigenetic alterations are dynamic in nature due to their frequent interaction with the environmental factors such as nutritional status, living conditions etc.[9,10] One of the epigenetic changes i.e. methylation of DNA occurs predominantly at the CpG island of the gene promoter. Promoter DNA methylation might have an important role in RHD pathogenesis; wherein change in methylation pattern may regulate the various genes detrimental in RHD. Further, few studies are available which explore the methylation pattern in RHD.[11,12] Endothelial cells line the cardiovascular system and are among the firsts in response to the stimuli such as flow alteration, anti-endothelial cell antibodies or environmental factors e.g. smoke by secretion of cytokines such as TNFα and interleukins. TNFα, IL-6, IL-1β, IFNγ, and IL-8 have been well characterized in inflammation and autoimmune processes. Investigations have also revealed the role of epigenetic regulation of endothelial gene expression.[13,14] Endothelial cells are believed to play crucial role in RHD as well, however lack of information regarding the regulation of gene expression from epigenetic perspective in endothelium dysfunction have hindered the understanding of RHD. Thus, we hypothesize that the mitral valve lining of endothelial cells may play a central role in RHD development by the alteration in physiological genomic and epigenomic signatures. The unveiling of the study may shed light on the current understanding and could append further insight into cryptic etiology of RHD.

Materials and methods

Ethical statement

The study was conducted after the ethical permission from the Institute Ethics Committee, Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, India (NK/653/Ph.D./14198). Written informed consent was collected prior to sample collection from the patient or the legal guardian.

Samples

Mitral valve tissue (n = 28) from chronic RHD patients undergoing valve replacement surgery were collected from Department of Cardiothoracic and Vascular Surgery, PGIMER. Age and sex matched control mitral valve (n = 22) were collected from autopsy cases with no sign of morphological or pathological heart disease. Mitral valve tissues (1–2 cm^2, 100–150 mg) were collected and immediately transported to the laboratory. Tissues were finely minced and kept overnight in RNA later™ (Ambion, USA) at 4 °C, and then stored at −80 °C till further use.

Blood samples (n = 37) were collected from the RHD patients visiting the Department of Cardiothoracic and Vascular Surgery, PGIMER, Chandigarh, India between 2015 and 2016 subjected to availability and consent of the patients. ARF (n = 14) blood samples were previously collected in Jai Vigyan mission mode (2000–2010, Indian Council of Medical Research, New Delhi) and stored at −80 °C. Umbilical cord (n = 10) was collected from the placental side of full term healthy mother immediately after their delivery from Department of Obstetrics and Gynaecology, PGIMER, Chandigarh, India. Age and sex matched control blood samples (n = 26) were collected from healthy volunteers without any history of autoimmune diseases, GAS infections, diabetes, or drugs consumption. For sera separation, blood was allowed to clot at room temperature followed by centrifugation at 1500 rpm for 5 min. Further, with the addition of 1x protease inhibitor (Sigma–Aldrich, USA) sera were stored at −80 °C till further use.

Culture of human micro vascular endothelial cells (HMVECs)

HMVECs of cardiac origin were obtained from Lonza (Switzerland). They were grown in endothelial cell basal medium (EBM)-2 supplemented with 5% fetal bovine serum (FBS), human recombinant epidermal growth factor

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