UNIVERSITI TEKNOLOGI MARA

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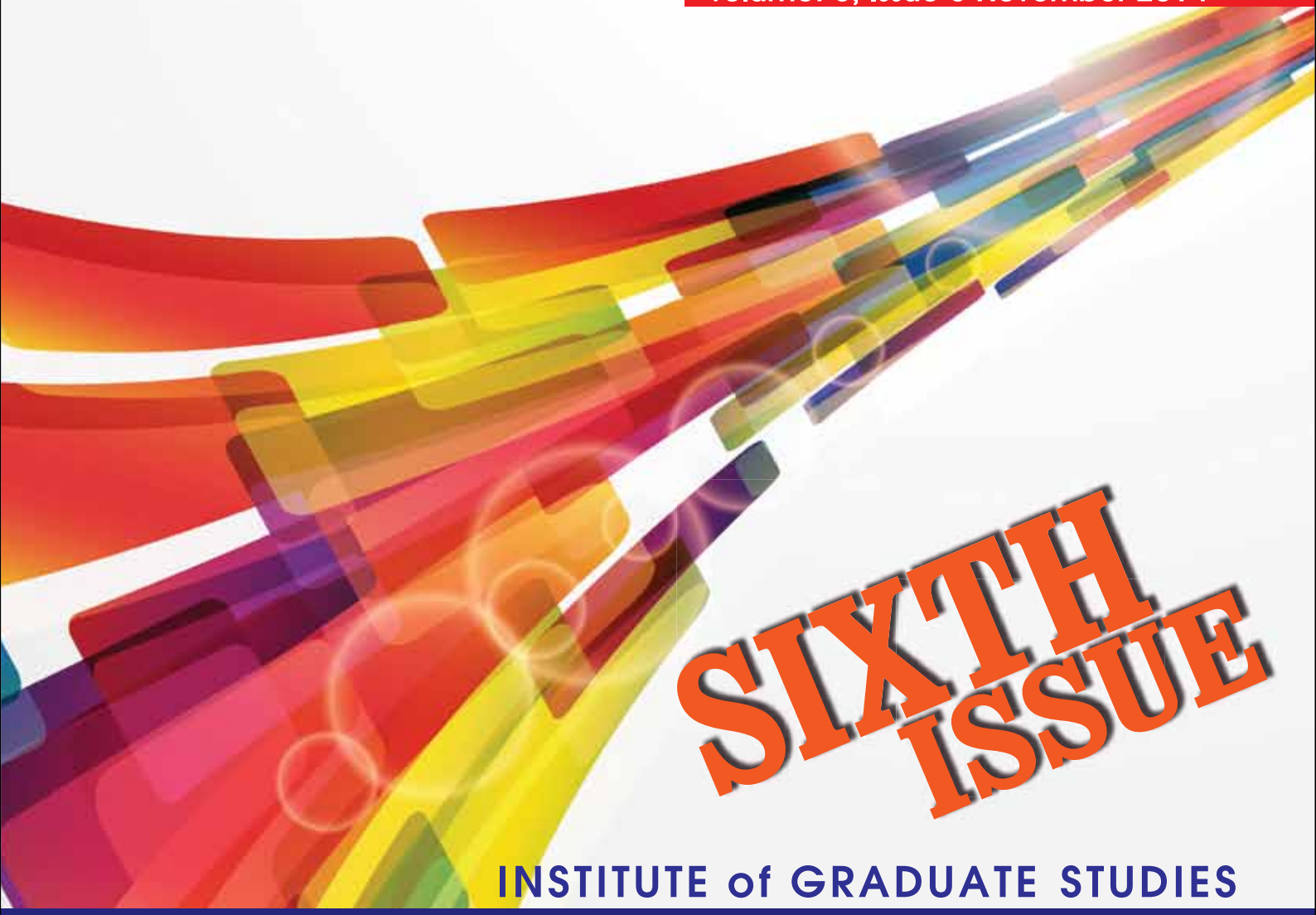

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Title :
Molecular Events Associated With Underlying Changes Of The Vascular Endothelium And Subendothelial Space During Atherogenesis In An Animal Model

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The development of atherosclerotic plaques is a multistep process involving changes in blood lipid composition, dysfunction of the endothelium, and infiltration of inflammatory cells. Cellular and molecular studies revealed enhanced expressions of several genes in development of atherosclerosis. This thesis aimed to investigate whether changed expressions of endothelial surface genes (VCAM, ICAM, and selectins), MCP-1, MMPs, and tissue inhibitor of MMPs (TIMPs) are associated with the underlying changes of the endothelium and subendothelial space in the development of atherosclerosis. In addition, the present study also determined whether any novel differentially expressed gene (DEG) is associated with atherogenesis. Rabbits were fed with 1 % cholesterol to induce atherosclerosis. Blood serum was collected for lipid profile analysis. Aorta tissues were used to study changes in morphology, ultrastructure, and gene expressions. Luminal endothelial surface from rabbit aortic tissue was examined by scanning electron microscopy (SEM) using low vacuum mode. The tissue cross-sections were stained with hematoxylin and eosin (H&E) for microscopic observations of intimal thickening. Total RNA was extracted from aorta tissues for gene expressions analysis. Differentially expressed genes (DEG, were analyzed by Real-time polymerase chain reaction (PCR) and Quantigene® Plex. Annealing

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control primer (ACP)-based GeneFishing™ PCR was used to analyze differentially expressed unknown genes. The DNA fragment from DEG was cloned, sequenced, and validated by Real-time PCR. Presence of highly expressed MMP genes in the intimal thickening of atherosclerotic tissues was detected using immunohistochemistry (IHC) staining. Lipid profiles obtained from rabbits fed with 1 % cholesterol showed highly significant difference ($p < 0.001$) in total cholesterol and low density lipoprotein (LDL) while terminating the study at week-2 and week-8. Ultrastructural observations of the aortic luminal surface by low vacuum mode SEM showed changes from normal regular smooth intact endothelium to irregular luminal surface including endothelial swelling and formation of 'craters' on the endothelial surface. In the present study, we examined the aorta tissues much closer to its natural conditions using a preparation not subjected to critical drying point and heavy metal coating. Ultrastructural changes of the luminal surface in atherogenesis indicate dysfunction of the endothelium. Higher expression of VCAM, P-selectin, E-selectin, and chemokine (MCP-1) might influence structural integrity of the luminal endothelium. H&E stained aorta tissues exhibited discernible intimal thickening at week-8 of atherogenesis; the tissues were found to be consisted of abundant foam cells. MMPs and TIMPs showed different expression profiles in Real-time PCR and Quantigene® Plex assays. Highest MMP-12 expression was detected by both assays at week-8 atherogenesis. IHC staining of the foam cells detected expressions of MMP -1, -3, and -12 in week-8 aorta tissues. We identified DEG detected from ACP-41 as cathepsin B gene; it was highly expressed at week-8 and week-12 of atherogenesis. Based on the findings of the present study, we can conclude that loss of endothelium integrity is associated with higher expressions of several types of endothelial surface genes. Additionally, we also found that intimal thickening was associated with differential expression profiles of MMPs and TIMPs genes. We also identified Cathepsin B as proatherogenic.