Circulating micro-particles as Potential biomarkers for cerebro-vascular ischemic infarction

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Abstract:

Cellular microparticles are plasma membrane vesicles mainly composed of lipids and proteins, which are released into the circulation by blood-cells and vascular cells during cellular activation or apoptosis. Microparticles are heterogeneous, differing in size, as well as in phospholipids and protein composition. In addition, microparticles display some specific cell surface proteins that indicate their cellular origin. Investigation into their biological activity has revealed diverse actions in coagulation, cell signaling and cellular interactions. These actions are mediated through their phospholipid rich surfaces and the expression of cell surface molecules which reflect their cell of origin and its state of activation .
Indeed, the numbers and characteristics of circulating microparticles have been found to be altered in many vascular diseases associated with an increased risk of both arterial and venous thrombosis. The complex role of microparticles in vascular accidents is an area of immense interest that promises to yield important advances into diagnosis and therapy. Few reports are available on role of circulating microparticles in thrombotic cerebral strokes. .
Currently, no practical, rapid and sensitive test is available for the diagnosis of acute ischemic stroke. A number of soluble molecules have been identified that are merely associated to these cerebrovascular accidents. Current knowledge from the field of cell-derived microparticles suggests that these membrane fragments may represent reliable biomarkers as they are cell-specific and are released early in the pathophysiological cascade of a disease.

Patients and methods: The study included 20 patients above 50 years of age experiencing focal neurological symptoms & signs lasting 24 hours or longer, with a relevant lesion within the brain as assessed by neurological imaging. All patients were recruited from the stroke unit of Kasr Al Aini hospital. All patients were subjected to full clinical evaluation, revision of their archived clinical progress reports , radiological and laboratory data. In addition assay of circulating microparticles for both quantitation and determination of cell of origin using flowcytometry technique was performed. The following fluorescent monoclonal antibodies were assayed : Endothelial : CD 62 E, Platelet : CD 61p, Monocyte : CD 14 and Erythrocyte : CD 235. The results of the patients were analyzed using SPSS-14 software and test selection for mean comparison depended on data distribution .

Results: Flowcytometry assay of circulating microparticles in studied cases revealed that the most common type of microparticles present in thrombotic stroke were platelet derived micropartcles (28.8%) and microparticles coexpressing both platelet and endothelial markers (39.5%), followed by MP with erythrocyte origin (19.2%), then those of monocytic origin (15%) and finally endothelial derived MP (2%). Comparing MP assay data in stroke patients versus controls revealed, a significantly higher CD 235 expression and highly significant greater expression of both CD 61p and CD 14 in stroke patients compared to controls. Coexpression of CD61p and CD62E was a common feature in stroke patients revealing a highly significant elevation in stroke patients compared to control subjects. Comparing MP assay coexpressing CD 62 E, CD 61p in patients with and without cardiac disease. there was a higher expression of these markers in stroke patients with cardiac disease compared to

stroke patients without cardiac disease. Regarding comparison of MP assay data in patients with and without Diabetes mellitus it showed higher CD 61p expression in stroke patients with diabetes. Similarly coexpressing of CD 62 E, CD 61p was significantly higher in DM. In comparison of MP assay data in patients not receiving anticoagulant therapy at time of sampling with controls there was significant higher expression of both CD 61p and CD 235 in patients compared to controls. Coexpression of CD61P and CD 62 E were compared in stroke patients versus controls. A highly significant higher levels of coexpresssion was noted in stroke patients. A cutoff value for the Coexpression of CD61p and CD62 E as a marker of thrombotic stroke was suggested to be **13.5%** using ROC curve statistical method. Other MP assay markers showed overlap between patients and control and calculation of cutoff values were not possible.

Conclusion: The most common type of microparticles present in thrombotic stroke is platelet derived micropartcles, and microparticles coexpressing both platelet and endothelial markers. Platelet drived MP are significantly higher in stroke patients with Diabetes mellitus. Coexpression of CD 62E and CD 61p is significantly higher in stroke patients with DM and with history of cardiac disease. MP coexpressing CD 62E and CD 61p can be used as a test for the early diagnosis of thrombotic stroke with high senstivity and specificity. Establishing a cutoff value for coexpression of CD 62E and CD 61p in stroke patients can contribute to the clinical applications as using MP assay in diagnosis of thrombotic propensity, monitoring of anticoagulant therapy, and detection of risk of stroke and ischemic heart disease in high risk patients.