

**IMMUNOTHROMBOSIS AND THE ROLE OF
NEUTROPHIL EXTRACELLULAR TRAPS
(NETS) IN THE PATHOGENESIS OF
ANTIPHOSPHOLIPID SYNDROME**

Thesis

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**التجلط المناعي و دور الكمائن الخارجية للكرات البيضاء متعددة النواه
□ في النشوء المرضى لمتلازمة مضادات الفوسفوليبيد**

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قالوا

لسببائك لا علم لنا
إلا ما علمتنا إنك أنت
العليم العظيم

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LIST OF ABBREVIATIONS

| | |
|-------------------|--|
| aCL | : Anti-cardiolipin |
| Ann | : Annexin |
| β2GPI | : β2glycoprotein i |
| APC | : Activated protein c |
| aPE | : Anti-phosphatidyl ethanolamine |
| aPLs | : Anti-phospholipid antibodies |
| ApoER2 | : Apoe receptor 2 |
| APS | : Anti-phospholipid syndrome |
| aPS | : Anti-phosphatidylserine |
| APS | : The anti-phospholipid syndrome alliance for clinical |
| ACTION | : trials and international networking |
| aPS/PT | : Anti-phosphatidylserine prothrombin |
| aPTT | : Activated partial thromboplastin time |
| C1-INH | : C1 esterase inhibitor |
| CAD | : Coronary artery disease |
| CCP | : Complement control protein |
| cfDNA | : Cell free dna |
| Cys | : Cysteine |
| DAMP | : Damage associated molecular pattern |
| DIC | : Disseminated intravascular coagulation |
| dRVVT | : Diluted russel viper venom time |
| DV | : Domain v |
| DVT | : Deep vein thrombosis |
| ECs | : Endothelial cells |
| GPIIb/IIIa | : Glycoprotein iib/iiia |
| H | : Histone |
| HK | : High molecular weight kininogen |
| HMGB1 | : High mobility group box 1 protein |
| HNPs | : Human neutrophil peptide |
| ICA | : Index of circulating anticoagulant () |
| ICAM | : Intracellular adhesion molecule |
| IFN | : Interferon |
| Ig | : Immunoglobulin |
| IL | : Interlukin |
| INR | : International normalized ratio |
| IP | : Induced protein |
| ISTH | : International society on thrombosis and haemostasis |
| KCT | : Kaolin clotting time |
| LA | : Lupus anticoagulant |

List of Abbreviations

| | |
|--------------------------------|--|
| LMWH | : Low molecular weight heparin |
| Lpa | : Lipoprotein a |
| MI | : Myocardial infarction |
| MPO | : Myeloperoxidase |
| NE | : Neutrophil elastase |
| NETs | : Neutrophil extracellular traps |
| NF-κB | : Nuclear factor-kappa beta |
| PAD4 | : Peptidylarginine deiminase 4 |
| PAMP | : Pathogen associated molecular patterns |
| PC | : Protein c |
| pDCs | : Plasmacytoid dendritic cells |
| PDI | : Protein disulfide isomerase |
| PDI | : Protein disulphide isomerase |
| PF4 | : Platelet factor 4 |
| PNP | : Pooled normal plasma |
| PS | : Phosphatidylserine |
| ROS | : Reactive oxygen species |
| SCT | : Silica test |
| SLE | : Systemic lupus erythematosus |
| ST | : Stent thrombosis |
| TF | : Tissue factor |
| TFPI | : Tissue factor pathway inhibitor |
| TLRs | : Tol-like receptors |
| TNF-α | : Tumor necrosis factor α |
| tPA | : Tissue plasminogen activator |
| tPA | : Tissue plasminogen activator |
| TXB2 | : Thromboxane β 2 |
| VEGF | : Vascular endothelial growth factor |
| VKA | : Vitamin k antagonists |
| VTE | : Venous thromboembolism |
| VWF | : Von willebrand factor |

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ABSTRACT

Background: Netosis is a complex process resulting in Neutrophil extracellular traps (NETs) release. It differs according to the stimulus and results in dramatic changes in the morphology of the cells. The exact stimulus that drives the cell in the direction of Netosis is still not clear. NETs have a procoagulant as well as an antifibrinolytic effect, if unregulated will result in thrombotic disorders as VTE and DIC. A link between NETs and the process of thrombosis have been suggested and role of antiphospholipid antibodies (especially B2GPI) in activating the process of Netosis in APS patients have been proposed.

Purpose: to demonstrate the presence of NETs and Histones in APS patients and their relation to different laboratory and clinical criteria mentioned in Sydney classification criteria.

Patients and Methods: 41 patients diagnosed as primary APS according to the Sydney classification criteria (2004) were recruited from our outpatients clinics. 40 normal age and sex matched controls were also recruited. Levels of NETs and Histones in sera of those subjects were measured and compared in patients and controls. Also NETs and Histones levels were compared against different laboratory criteria (Lupus anticoagulant, diluted Viper Venom Test, B2GPI and ACL) and clinical picture. Patients were divided into different groups according to positivity of different laboratory tests, levels of NETs and Histones were compared in those different groups.

Results: Both NETs and Histones levels were significantly higher in APS patients than in controls. Levels of NETs and Histones were significantly higher in triple positive patients compared to double and single positive patients.

Conclusion: NETs and histones were found to be correlated to APS suggesting a close relation to its pathogenesis as well as being related to laboratory positivity of those patients.

Keywords: NETs – Histones- Antiphospholipid syndrome

INTRODUCTION

Immunothrombosis is a term coined by *Engelmann and Massberg*; which describes an immune reaction induced by the formation of microthrombi; it is evident in both physiological and pathological settings in vivo (*Engelmann and Massberg, 2013*).

Immunothrombosis mediates the recognition of pathogens and damaged cells, and inhibits pathogen dissemination and its survival. Immunothrombosis can be viewed as a novel element of intravascular immunity, which is a part of the immune system that helps the host detect and eliminate pathogens in the vasculature (*Hickey and Kubes, 2009*).

Both arterial and venous thromboses involve hemostatic mechanisms that depend on active participation of cells of the innate immunity (mainly monocytes and neutrophils). These cells cause platelet activation, fibrin formation and propagation during the development of thrombosis (*Engelmann and Massberg, 2013*).

Neutrophils are one of the major components of immunothrombosis through neutrophil extracellular traps

(NETs). NETs act as catalytic surfaces that promote and compartmentalize the coagulation system (*Massberg et al., 2010*).

NETs were first described as a part of innate immunity that binds microorganisms, prevents them from spreading, and ensures a high local concentration of antimicrobial agents to degrade virulence factors and kill bacteria (*Brinkmann et al., 2004*).

NETs contains proteins (elastase, cathepsin G, and myeloperoxidase) from azurophilic granules and proteins from specific granules and tertiary granules (lactoferrin and gelatinase) respectively. DNA and histones are also a major structural component of NETs (*Brinkmann et al., 2004*).

NETs provide matrix for fibrin deposition, platelet entrapment and subsequent activation (*Kambas et al., 2012*).

The components of NETs provide procoagulant activities: DNA initiates the intrinsic pathway via the activation of factor XII, histones are strong generators of thrombin (*Von Bruhl et al., 2012; Iba et al., 2014*) and granule proteins contribute to suppression of the anticoagulation system via degradation of tissue factor

pathway inhibitor (TFPI) and probably thrombomodulin (*Iba et al., 2014*).

The antiphospholipid syndrome (APS) is an acquired autoimmune condition characterized by both arterial and venous thrombosis, gestational morbidity and presence of continuously elevated serum titers of antiphospholipid antibodies (aPL) (*Saleem, 2014*).

Despite an abundance of clinical and basic research, pathophysiology of APS remains an enigma to this day (*Chaturvedi and McCrae, 2015*).

Proposed pathogenic mechanisms include increased oxidative stress, impaired functions of nitrous oxide systems (NOS), activation of receptors by beta 2 glycoprotein -1 antibody (B2GP-1 Ab), increased expression and activation of tissue factor (TF), increased free thiol form of factor II, disruption of the annexin A5 shield, antibody mediated activation of complement C3 and C5 and increased expression of Toll like Receptor (TLR) 7 and TLR 8 (*Giannakopoulos and Kirilis, 2014*).

Clinical presentation of APS is more polymorphic than it was thought (*Öztürk et al., 2004*) making its diagnostic criteria an ongoing process that is modified as