Apoptosis and abnormal phagocytic function of neutrophils in chronic uraemic patients

Thesis

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Submitted by

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Abstract

The increased incidence of bacterial infections among patients with renal failure suggests that "professional" phagocytes such as neutrophils are functionally impaired. This dysfunction is multifactorial and not fully understood and has been ascribed to uremic toxins, hemodialysis and others.

The aim of the current study was to evaluate apoptosis and phagocytosis in uremic neutrophils; neutrophils of hemodialysis patients and those who are not dialyzed yet, and apoptosis inducing activity of uremic plasma and to investigate the correlation between apoptosis and phagocytic function of neutrophils in uremic patients.

The study was conducted on forty patients ranging (40-60) years old and divided into four groups; 10 chronic hemodialysis patients for 26.2 months, 10 chronic renal failure patients not hemodialyzed yet, 10 normal healthy volunteers as a control group, and 10 normal healthy volunteers as a control group for normal healthy volunteers.

Neutrophils from hemodialysis patients, non hemodialysis patients and matched healthy volunteers were processed for quantification of apoptosis and phagocytosis.

The neutrophils from different groups were incubated for 24 hours in culture media supplemented with either autologous or heterologous plasma from matched pairs from other groups. After the incubation period, neutrophils aliquots were processed for quantification of apoptosis and phagocytosis.

We found that neutrophils of non-dialysis patients exhibited a significant higher apoptosis rates than neutrophils from hemodialysis patients and normal healthy volunteers. Also, neutrophils of hemodialysis patients exhibited a significant higher apoptosis rates than neutrophils of normal healthy volunteers. Moreover, plasma of non-dialysis patients is more apoptogenic than hemodialysis patients and normal healthy volunteers. Also, plasma of hemodialysis patients is more apoptogenic than normal healthy volunteers. Also, we observed that normal neutrophils of hemodialysis patients exhibited a significant lower phagocytosis rates than neutrophils of non-dialysis patients and normal volunteers. Also, neutrophils of non-dialysis patients exhibited a significant lower phagocytosis rates than normal neutrophils. Our study showed that plasma of non-dialysis patients has more suppressive effect on phagocytosis than plasma of hemodialysis patients and normal healthy volunteers. Also, plasma of hemodialysis patients has more suppressive effect on phagocytosis than plasma of normal healthy volunteers. But, neutrophils of hemodialysis patients are more prone to suppressive effect on phagocytosis than non-dialysis patients and normal healthy volunteers. While, neutrophils of non-dialysis patients are more prone to suppressive effect on phagocytosis than neutrophils of normal healthy volunteers. We concluded that there is significant inverse correlation between apoptosis and phagocytosis in all studied groups. This suggests that there is significant inverse correlation between neutrophil apoptosis and neutrophil functions. This suggests that apoptotic neutrophils are dysfunctional.

Key words: Apoptosis, phagocytosis, neutrophils, hemodialysis, chronic renal failure and non-hemodialysis.

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Abbreviations

- **A1:** Antagonist of apoptosis. A Bcl-2 family member that is also known as BFL-1 protein.
- **ADP:** Adenosine diphosphate.
- **AIF:** Apoptosis inducing factors.
- **Akt-1:** Active protein kinase B-alpha.
- **ANT:** Adenine nucleotide translocator.
- **ANT:** Nucleotide translocase.
- **APAF:** Apoptosis protease activating factor.
- **APCs:** Antigen-presenting cells.
- **APO:** Apoptosis
- **APO-1:** Also known as Fas and CD95, a member of the TNF receptor family.
- **APO-1L:** APO-1 ligand, also known as fas ligand. A member of the TNF family that induces apoptosis.
- **APO-2:** Receptor for TRAIL/APO-2L. A member of the TNF receptor family.
- **APO-2L:** APO-2 ligand that is also known as TRAIL. APO-2L is a member of the TNF family that induces apoptosis.
- **APO-3:** Death domain containing receptor. Also known as DR3, WSL-1, TRAMP or LARD
- **APO-3L:** The ligand for the death-domain-containing receptor Apo-3. It induces apoptosis.
- **ARC:** Apoptosis repressor with caspase recruitment domain.
- **ARF:** Acute renal failure.
- **ATP:** Adenosine trihosphate.

- **Bad:** Bcl-xL/Bcl-2 associated death promoter.
- **Bak:** Bcl-2 antagonist/killer.
- **Bax:** Bcl-2 associated x protein.
- **BC:** Before Christ.
- **Bcatenin:** aka plakoglobin.
- **Bcl-2:** B-cell lymphoma gene 2 product.
- **Bcl-x:** A Bcl-x isoform that inhibits apoptosis
- **Bcl-XL:** Long version of Bcl-2- related gene product X.
- **Bcl-xS:** Short form of Bcl-x.
- **BDNF:** brain derived neurotrophic factor.
- **BH:** BCL-2 homology domains designated BH1, BH2, BH3, and BH4.
- **BID:** BH3 interacting domain death agonist.
- **Bik:** Bcl-2 interacting killer.
- **Bim:** A member of the Bcl-2 family that promotes apoptosis.
- **BOD:** Bcl-2-related ovarian death gene.
- **Bok:** Bcl-2 related ovarian killer.
- **C. elegans:** Caenorhabditis elegans.
- **C1q:** First Component of Complement, q portion.
- **C3bi:** Thirdt Component of Complement, bi portion.
- **C5a:** Fifth Component of Complement, a portion.
- **Ca^{2+}:** Calcium.
- **CAD:** Caspase-activated DNase.
- **cAMP:** cyclic Adenosine monophosphate.
- **CAPD :** Continuous ambulatory peritoneal dialysis.
- **CARD:** Caspase activation and recruitment domain.
- **Caspases:** cysteine aspartate-specific proteases
- **CAV:** Chicken anaemia virus.

- **CC:** First two cysteine (C) amino acid resiues are in juxta position. It belongs to β-chemokine family. They are more specific towards monocytes.
- **CD14:** Cluster of Differentiation 14.
- **CD36:** Cluster of Differentiation 36.
- **CD95 (Fas/Apo-1):** Cluster of Differentiation 95. Also known as Fas and APO-1, a member of the TNF receptor family
- **CED:** Caenorhabditis Elegans Death product.
- **CED-3:** Caenorhabditis Elegans Death 3. A cell death gene in the nematode C. elegans. CED-3 promotes apoptosis and is a homologue to Apaf-3/Caspase-9 and Caspase-3.
- **cFLIPs:** Cellular homologues of viral FADD-like ICE inhibitory protein).
- **c-fos**: This ~62 kDa leucine zipper protein cannot homodimerize but rather functions in heterodimeric complex with c-jun and other members of the AP1 family of transcription factors.
- **cIAP1:** Cellular inhibitor of apoptosis protein 1
- **cIAP2:** Cellular inhibitor of apoptosis protein 2.
- **CIN:** Ghronic interstitial nephritis.
- **c-jun:** This proto-oncogene encodes a ~45 kDa transcription factor that is a member of AP1 family of transcriptional proteins. c-jun must form dimers to function and does so through the leucine zipper motif. A second partner, usually c-fos, generates the transcriptionally active heterodimer.
- **CK:** Creatine kinase.
- **CK-18:** Cytokeratin-18,
- **CML:** Chronic myelogenous leukaemia.
- **c-myc:** Myelocytoma oncogene.
- **CORE:** Circulating opsonin receptor expression.

- **cPLA2:** Cytoplasmic phospholipase A2.
- **CPP32:** Also known as Caspase-3.
- **CR3:** Receptor of the third component of complement.
- **CRF:** Chronic renal failure.
- **CrmA:** Cytokine response modifier A.
- **Cr-P:** Creatine phosphate.
- **CsA:** Cyclosporin A.
- **CSF:** Colony stimulating factors.
- **C-terminus:** Carboxy terminus.
- **CTLs:** Cytotoxic T lymphocytes
- **CU:** Cuprophane membranes.
- **CXC:** First two cysteine (C) amino acid residues separated by a separate amino acid (X). It belongs to α-chemokine family. They are more specific towards neutrophils.
- **CycD:** Cyclophilin D.
- **cyt c:** Cytochrome c.
- **D4-GDI:** Rho GDP-dissociation inhibitor, D4. GDP-Dissociation Inhibitor for Ras-related Rho family GTPase.
- **dATP:** deoxyadenosine triphosphate.
- **DcR:** Decoy receptor.
- **DcR-1(TRID/TRAIL-R3/LIT):** Decoy receptor 1. DcR-1 is a receptor for TRAIL that inhibits TRAIL signaling. DcR-1 is also known TRID, LIT and TRAIL-R3.
- **DcR-2 (TRAIL-R4/TRUNDD):** Decoy receptor 2. DcR-2 is the receptor for Trail that inhibits TRAIL signaling. DcR-2 is also known as TRUNDD and TRAIL-R4.
- **DcR-3:** Decoy receptor 3. It binds to FasL and inhibits FasL-induced apoptosis.

- **DD:** Death domain.
- **DED:** Death effector domain.
- **DEVD:** Amino acid residues (Asp-Glu-Val-Asp) of Caspase-3 cleavage site within PARP.
- **DFF45/ICAD:** 45 kDa component of DNA fragmentation factor; inhibitor of the caspase-activated deoxyribonuclease.
- **Diablo:** Direct inhibitor of apoptosis protein [IAP] binding protein with low isoelectric point [pI].
- **DIP I:** Degranulation inhibiting protein I.
- **DIP II:** Degranulation inhibiting protein II.
- **DISC:** Death-inducing signaling complex.
- **DMTU:** dimethylthiourea.
- **DNA:** Deoxyribonucleic Acid.
- **DNA-PKCS:** DNA protein kinase catalytic subunit.
- **DNA-RFC140:** 140 kDa subunit of DNA replication factor C.
- **DND:** Delayed Neuronal Death.
- **DR:** Death receptor.
- **DR-3 (Apo-3, LARD, TRAMP, Wsl-1, Tweak):** Death receptor 3. DR-3 is a member of the TNF receptor family that is also known as Apo-3, WSL-1, TRAMP or LARD.
- **DR-4 (Apo-2/TRAIL-R1):** Death receptor 4. One of the receptors for TRAIL that is also known as APO and TRAIL-R1.
- **DR-5 (TRAIL-R2/TRICK-2/KILLER):** Death r receptor 5. One of receptors for TRAIL that is also known as TRAIL-R2, TRICK-2 and KILLER.
- **DR-6:** Death receptor 6.
- **EAE:** Experimental autoimmune encephalitis.
- **EC:** Endothelial cell.

- **EGF:** Endothelial growth factor.
- **EIA:** Enzyme Immunoassay.
- **ELC:** Epstein-Barr virus-induced molecule 1 ligand chemokine (CCL19).
- **ENA-78:** Epithelial cell-derived neutrophil attractant-78.
- **ER:** Endoplasmic reticulum.
- **ERICE:** A FLICE-activatable caspase. Also known as caspase-13.
- **ERK:** p42/44 extracellular signal-related protein kinase.
- **ESRD:** End stage renal disease.
- **FADD:** Fas-associated death domain.
- **FAK:** focal adhesion kinase.
- **Fas (Apo-1, CD95):** Fas is a member of the TNF receptor family promotes apoptosis. Also, it is known as CD95 and APO-1.
- **FasL:** Fas ligand.
- **Fc:** Fragment crystallizable (Portion of antibody molecule bound by membrane receptors).
- **Fcγ:** Fragment crystallizable (Portion of antibody molecule bound by membrane receptors), portion gamma
- **FcγRIIa (CD32):** Receptor for fragment crystallizable (Portion of antibody molecule bound by membrane receptors), portion IIa.
- **FcγRIIIb (CD16):** Receptor for fragment crystallizable (Portion of antibody molecule bound by membrane receptors), portion IIIb.
- **FD:** Factor D.
- **FLICE:** Fadd-like ICE. FLICE is also known as Caspase-8.
- **FLICE-2**: Fadd-like ICE. FLICE is also known as Caspase-8.
- **FLICE-2:** Fadd-like ICE-2. FLICE-2 is also known as Caspase-10.
- **FLICE-2:** Fadd-like ICE-2. FLICE-2 is also known as Caspase-10.
- **FLIP:** Fadd-like ICE-2. FLICE-2 is also known as Caspase-10.
- **fMLP-M:** formyl-methionyl-leucyl-phenylalanine methylester.

- **Gas2:** Growth arrest specific gene product 2.
- **GCP-2:** Granulocyte chemotactic protein.
- **G-CSF:** Granulocyte colony-stimulating factor.
- **GDI:** GDP dissociation inhibitor.
- **GIP I:** Granulocyte inhibiting protein I.
- **GIP II:** Granulocyte inhibitory protein II.
- **gln22/ser23: Gln** = glutamine; **Ser** = Serine.
- **Glucose-6-P:** Glucose-6-phosphate (glucose-6-P),
- **GM-CSF:** Granulocyte macrophage colony-stimulating factor.
- **gp:** Glycoprotein.
- **GRO-α:** Growth-related oncogene alpha.
- **GRO-β:** Growth-related oncogene beta.
- **GRO-δ:** Growth-related oncogene delta.
- **GVHD:** Graft versus host disease.
- **H_2O_2:** Hydrogen peroxide.
- **HCV:** Hepatitis C virus.
- **HDx:** hemodialysis.
- **HIAP1:** Human inhibitor of apoptosis protein 1.
- **HIAP2:** Human inhibitor of apoptosis protein 2.
- **HIV:** Human immunodeficiency virus.
- **HK:** Hexokinase.
- **HILP:** Human IAP-like protein, regulates programmed cell death downstream of Bcl-xL and cytochrome c. Also known as XIAP.
- **HMS:** hexose monophosphate shunt.
- **HnRNP**: heteronuclear ribonucleoproteins.
- **Hsp90:** 90 kDa heat shock protein.
- **HTN:** Hypertension.
- **IAPs:** Inhibitors of apoptosis proteins.

- **iC3b:** Inhibitory of third complement component 3b.
- **ICAD:** Inhibitor of caspase-activated DNAse.
- **ICAM:** Intercellular adhesion molecule.
- **ICAM-3:** Intercellular adhesion molecule 3.
- **ICE:** Interleukin-1beta converting enzyme.
- **ICE-LAP3:** ICE-like apoptotic protease 3. ICE-LAP3 is also known as Caspase-7.
- **ICE-LAP6:** ICE-like apoptotic protease 6. ICE-LAP6 is also known Caspase-9.
- **ICErel II:** ICE/CED-3-related protease. ICErel II is also known as Caspase-4.
- **ICErel III:** ICE/CED-3 related protease. ICErel III is also known Caspase-5.
- **Ich-1:** ICE and ced-3 homologue-1. Ich-1 is another name for Caspase-2.
- **Ich-2:** Ice and ced-3 homologue-2. Ich-2 is also known as Caspase-4.
- **Ich-3:** ICE and ced-3 homologue-1. Ich-1 is another name for Caspase-11.
- **IETD:** Ile-Glu-Thr-Asp. The amino acid sequence corresponds to one of the Caspase-8 cleavage sites (amino acids 172-175) of the inactive caspase-3 precursor.
- **IgA:** Immunoglobulin A.
- **IgG:** Immunoglobulin G.
- **IgM:** Immunoglobulin B.
- **IkB:** Inhibitor of NF-kB.
- **IL:** Interleukin.
- **IL-1β, -2, -3, -6, -8, -10, -12 and -15:** Interleukins 1 beta, 2, 3, 6, 8, 10, 12 and 15.
- **IL-IRa:** Interleukin I receptor antagonist.