Original Articles

LYMPHOCYTE APOPTOSIS AND ADVERSE PREGNANCY OUTCOME

Israa F. Al-Samarai*MBChB, M.Sc. Ph.D.*, **Malka Al-Sadi****MRCOG*, **Ibtisam Al-Ubusi*****B.Sc., M.Sc., Ph.D.*,**

Abstract

Background: Apoptotic cell death plays an important role in cell biology and pathology including studies of embryonic development, pathogenesis of diseases, and the response of cell to therapy.

Aim of the study: To clarify the relation between apoptosis and oxidative stress in different pregnancy outcome, namely miscarriage and PET.

Patient and methods: The study involved 30 pregnant women; 10 were preeclampsia, 10 had miscarriage (before 20 weeks gestation).
Surface morphological changes of lymphocyte apoptosis were diagnosed by phase contrast and Interference contrast microscopy. Lipid peroxidation using thiobarbituric acid reactive species (malondialdehyde). Erythrocyte glutathione was estimated by Lang *et al* method, Zinc and Copper were estimated using atomic absorption spectrophotometer. Statistical analysis done using unpaired student T- test, and correlation coefficient.

Results: We found a significant increase in lymphocyte apoptosis in preeclamptic patients ($p<0.05$) than normal pregnant, the increase in MDA level was highly significant $p<0.005$ the same thing applied to consumption of glutathione in preeclamptic patients $p<0.005$ .there was also positive correlation between increased apoptotic process and oxidative stress variables. In miscarriage there was a significant increase in lymphocyte apoptosis compared to normal pregnant ($p<0.05$) also there was strong positive correlation between apoptotic process and oxidative stress ($r=0.92$).

Conclusion: Lymphocyte apoptosis and oxidative stress was significantly increased in PET and miscarriage; this means that oxidative stress can induce PET and miscarriage in pregnant women.

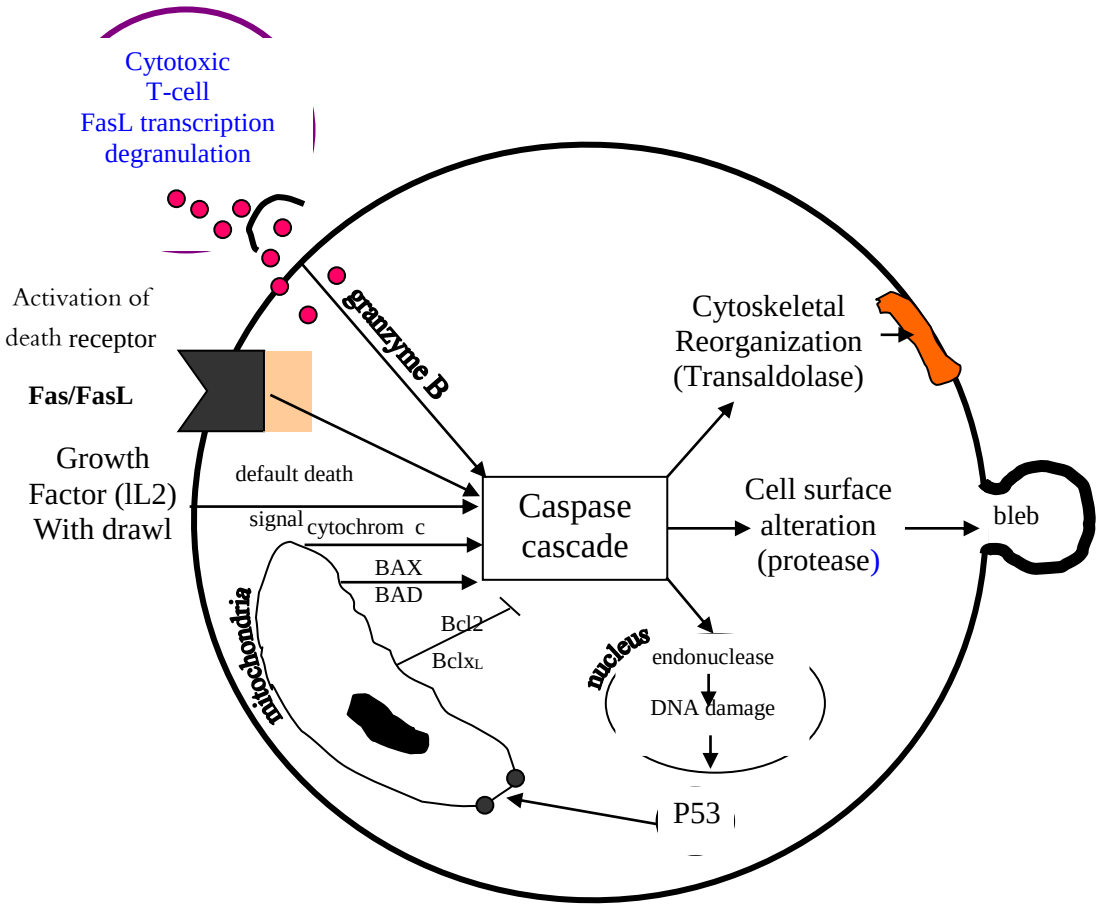
Key words: apoptosis, oxidative stress, PET, miscarriage

Iraqi J Med Sci, 2004; Vol. 3 (1):3-7

Introduction

Apoptotic cell death plays an important role in cell biology and pathology including studies of embryonic development, pathogenesis of diseases, and the response of cell to therapy[1].
Apoptosis is a distinct type of cell death in which an individual cell undergoes an internally controlled transition from an intact metabolically active state into a shrunken remnant retaining their membrane integrity[2]. The internal organelle does not undergo lysis during apoptosis and little leakage of the contents of the dying cell can be detected so apoptotic cell death does not induce an inflammatory response. Instead the shrunken apoptotic bodies are phagocytosed by macrophages and their contents are recycled, therefore apoptosis provide the organism with a safe ,clean method to remove dying cells without evoking an inflammatory response[3] this diagram summarizes a small portion of the apoptosis regulation pathway that have recently been delineated[4].

* Dept. Clinical Physiology, ** Dept. Gyneacology & Obstetrics, *** Dept. Chemistry & Biochemistry, College of Medicine, Al-Nahrain University.
Address Correspondence to Dr. Israa F. Al-Samarai.
Received 28th December 2002: Accepted 8th November 2003.



Summary of some factors affecting apoptosis

Apoptotic cells has distinct morphological features these are; membrane bleb formation membrane spikes, nuclear shrinkage with chromatin condensation, ordered cleavage of DNA, compactness of cytoplasmic organelles and lastly disintegration of cell into apoptotic bodies[5].

Apoptosis can be induced by variety of stimuli as ionizing radiation, cytotoxic drugs, and reactive oxygen species (ROS) including free radicals[6]: free radicals are any species that contain one or more unpaired electron(s)[6]. These ROS are highly reactive chemicals that are produced during participation of oxygen in redox reaction in normal metabolic pathway can directly penetrate cell membrane and induce mitochondrial permiabilization then release of cytochrome C which activate apoptosis[7], these ROS are able to cleave DNA and activate certain enzymes as endonuclease and phospholipase[8,9]. Previous work in our lab had shown that apoptosis is associated with generation of free radicals.

In contrast zinc inhibits apoptosis at three levels: at nuclear level by suppressing endonuclease enzyme[10], at cytosole level by inhibition of caspase activation[11], at mitochondrial level by increase in Bcl2/Bax ratio, also Zn is involved in scavenger ability of superoxide dismutase[12]. Ceruloplasmin is one of the scavengers against oxidative stress[13].

The aim of this work is to clarify the relation between apoptosis and oxidative stress in different pregnancy outcome.

Subjects & Methods

Thirty pregnant women were enrolled in this prospective study. The mean age was (29±1.23). Ten were preeclampsia (after 20 week gestation). Ten had miscarriage (before 20 weeks gestation) and 10 were normal pregnant. Preeclampsia was defined as blood pressure of 140/90mmHg or more occurs after 20 weeks gestation in a previously normotensive lady with protein urea of 300mg/dl or more. Miscarriage was defined as percentage loss before 20 weeks gestation. Beside the routine investigation each patient had the following tests: First: 2 ml of anticoagulated blood was processed for lymphocyte separation, lymphocyte layer was separated using Ficoll 400

(pharmacia Fine Chemicals) washed three times with phosphate buffered saline (PH 7.2) then lymphocytes counted by Neubaur counting chamber, then the non viable lymphocytes were excluded by trypan blue exclusion test (Figure 1). Surface morphological changes of lymphocyte apoptosis were diagnosed by phase contrast and Interference contrast microscopy[14] (Polyvar Richert-Jung microscope), cell morphology regarded as the most specific hallmark of apoptosis[15]. Second: blood samples were processed for estimation of lipid peroxidation using thiobarbituric acid reactive species(MDA) according to (Satoh)[16], erythrocyte glutathione was estimated by Lang **et al** method[17], Ceruloplasmin which is an acute phase protein was estimated using spectrophotometric method of Menden *et al*[18], Zinc and Copper were estimated using atomic absorption spectrophotometer.

Statistical analysis done using unpaired student T- test, and correlation coefficient.

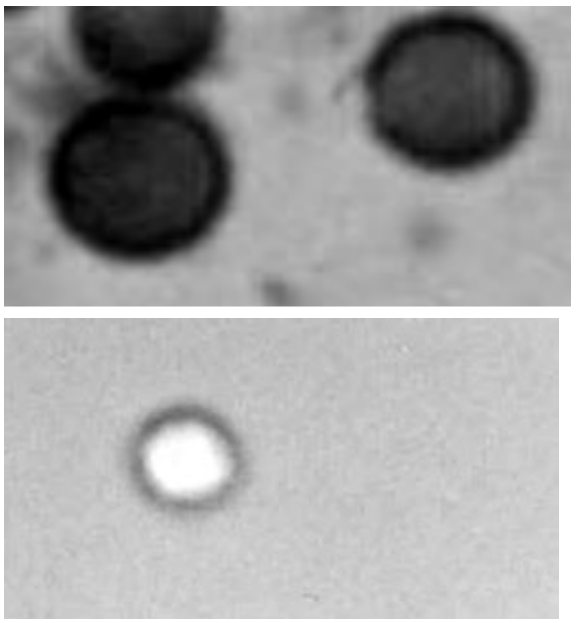


Figure 1: Lymphocyte viability assessed by trypan blue exclusion test; viable cells exclude the deye (bottom; while non viable cells stain with trypan blue (dark cell top)

Results

Morphological changes of lymphocyte apoptosis including membrane bleb formation, protrusion of echinoid spikes and lastly disintegration of cells into apoptotic bodies are shown in figure 2.

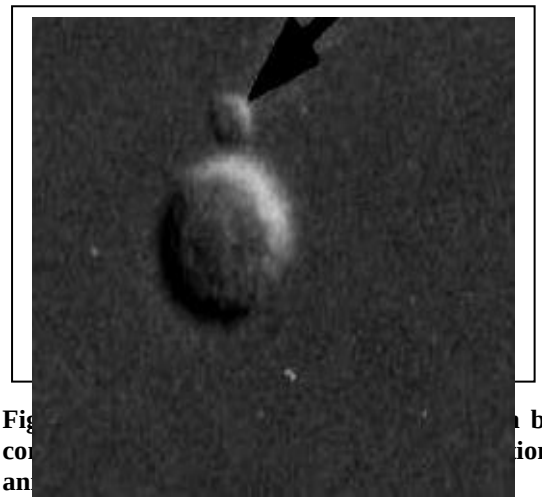


Fig … by phase co… …ion (x 400 an…

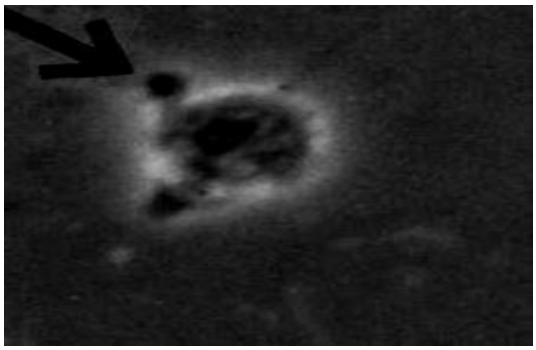
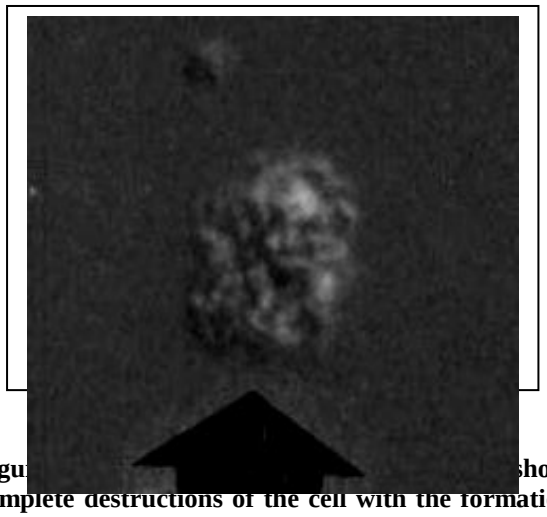


Figure 2: Peripheral blood lymphocyte showing multiple bleb formation (x 400 annular diaphragm)



Figu… …showing complete destructions of the cell with the formation of apoptotic bodies: (x400annulardiaphragm)

As shown in the table 1 there was significant increase in lymphocyte apoptosis in preeclamptic ($p<0.05$) than normal pregnant, the increase in MDA level was highly significant $p<0.005$ the same thing applied to consumption of glutathione in preeclamptic patients $p<0.005$ .there was also positive correlation between increased apoptotic process and oxidative stress variables (MDA, thiol, Zn and Cu). These results simulate those seen by Diedrich in 2001.

In miscarriage there was also significant increase in lymphocyte apoptosis compared to normal pregnant ($p<0.05$) also there was strong positive correlation between apoptotic process and oxidative stress ($r=0.92$).

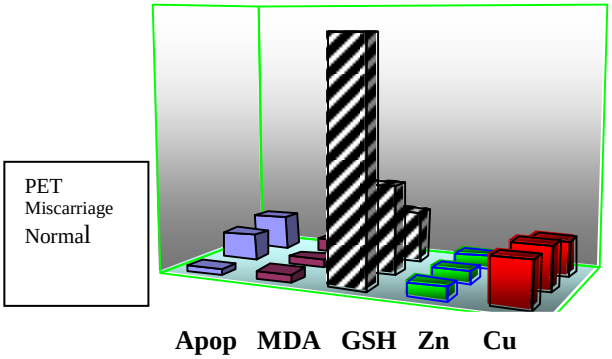


Figure 3: Over all changes associated with PET and Miscarriage; Cu,Zn,GSH, MDA, and %apoptotic lymphocyte .

Table1: Changes in % apoptotic cells and oxidative stress variables compared in normal pregnants miscarriage and PET

N =30	Normal	Miscarriage	P*	PET	P*
Apop. Cells %	3.02±0.35	15±2.52	Sig	19.58±3.96	Sig
MDA (mg/dl	4.7±1.09	4.36±2.31	ns	7.3±2.33	Sig**
GSH µm/L	136±48.3	50.2±10.04	Sig	28.8±8.76	Sig**
Zn mg/dl	0.75±0.10	0.74±0.04	ns	0.7±0.09	ns
Cu mg/dl	25.7±8.66	27.16±4.33	ns	19.58±6.96	ns

ns = non significant * = P<0.05, ** P <0.005

Discussion

An important prerequisite for a successful pregnancy is that maternal immune system does not reject the fetus, down-regulation of T-helper 1 (TH_1) associated cellular immune response could therefore be essential[20].

Apoptosis has been shown to regulate immunological over-reactivity and the level of TH_1 cells[20]. Fournel, et al had found enhanced CD95 ligand expression in peripheral blood lymphocyte suggesting that it may act as immunomodulator during pregnancy[21]. Elevated serum soluble Fas are associated with preeclampsia; such elevation might indicate protection of maternal T-lymphocyte apoptosis and consequently lead to the maternal immune intolerance noted in preeclampsia[22].

In the present study we found that lymphocyte apoptosis is significantly increased in cases of miscarriage as well as in pre-eclampsia (15% & 19% respectively compared to 3% in normal pregnancy). Also there was significant decrease in glutathione level in both preeclampsia and miscarriage which is in line with increased oxidative stress in both conditions[23], but we could not find significant difference in zinc and copper.

Daunter in 1992 has found controversial results regarding the total counts of T cell subset in the peripheral blood[23].

Gunter in 1998 found that the TH_1/TH_2 cytokine ratio in T cell of women during pregnancy and after delivery was significantly decreased[20]. In contrast the TH_1/TH_2 ratio was elevated to near normal in women with recurrent spontaneous abortion[20].

Apoptosis in decidual and villous cells have been studied in different pregnancy complications; Chiu in 2001 concluded that apoptosis activity in hydatidiform mole might be considered as a prognostic indicator for predicting the clinical behavior[24]. Li *et al* in 1999 concluded that mifepristone and misoprostol used for terminating human early pregnancy induce decidual and villous cells excessive apoptosis[25]. Qumsiyeh 2000 found that apoptosis of the stromal cells and cell proliferation in blood vessels and stoma play an important role in the differentiation and functioning of villi and that these changes could explain the etiology of spontaneous abortion and growth retardation of chromosomally abnormal embryos[26]. Shiraishi 1996 suggested that placental apoptosis caused by activation of maternal cytotoxic T lymphocytes may play important roles in the rejection of fetal allographts[27]. Fortunato 2000 suggested apoptosis as a possible pathway to metalloproteinase activation and fetal membrane degradation in premature rupture of membrane[28].

References

1. Wyllie, A.H.: Cytochemical; method for detection of apoptosis. J Histochem Cytochem, 1992; 47: 1101-9.
2. Kerr, J.F.R., Wyllie, A.H., and Currie, A.R.: Apoptosis: A basic biological phenomenon with wide ranging implication in tissue kinetics. Br J Cancer, 1972; 26: 239-57.

3. Ohta, K., and Yamashita, N.: Apoptosis of eosinophils and lymphocytes in allergic inflammation. J Allergy Clin Immunol, 1999; 104: 14-21.
4. Barrett, K.L., Willingham, J.M., Graven, A.J., and Willingham, M.C.: Advances in cytochemical methods for detection of apoptosis. J Histochem Cytochem, 2001; 49: 821-32.
5. Collins, J.A., Shandl, C.A., Young, K.K., Vesiey, J., and Willingham, M.C.: Major DNA fragmentation a late event in apoptosis. J Histochem Cytochem, 1997; 45: 923-34.
6. Reed, J.C.: Mechanisms of apoptosis. Am J Pathol, 2000; 157: 1415-30.
7. Dumont, A., Hehner, S.P., Hofmann, T.G., Ueffing, M., Droge, W., and Schmitz, M.L.: Hydrogen peroxide-induced apoptosis is CD95-independent requires the release of mitochondria-derived reactive oxygen species and the activation of NF-Kappa B. Oncogene, 1999; 18: 747-57.
8. Cregan, S.P., Smith, B.P., Brown, D.L., and Mitchel, R.E.: Two pathways for the induction of apoptosis in human lymphocytes. Int J Radiat Biol, 1999; 75(9): 1069-86.
9. Cotran, R.S., Kumar, V., And Collins, T.: Cellular Pathology:Cell injury and cell death. In robbins Pathologic basis of disease. 6th edition, W.B. Saunders Company, 1999; pp12-25.
10. Powell, S.R.: The antioxidant properties of zinc. J Nutr, 2000; 130: 1447s-54s.
11. Truong-Tran, A.Q., Ho, L.H., Chai, F., and Zalewski, P.D.: Cellular zinc fluxes and the regulation of apoptosis/gene-directed cell death. J Nutr, 2000; 130: 1466s-69s.
12. Pena, M.M.O., Lee, J., and Thiele, D.J.: A delicate balance: homeostatic control of copper uptake and distribution. J Nutr, 1999; 129: 1251-60.
13. Linder, M.C., and Azam, M.H.: Copper biochemistry and molecular biology. Am J Clin Nutr, 1996; 63: 797s-811s.
14. Willingham, M.C.: Cytochemical methods for the detection of apoptosis. J Histochem Cytochem, 1999; 47: 1101-9.
15. Zamzani, N., Kroemer, J.: Condensed matter in cell death. Nature, 1999; 401: 127-8.
16. Satoh, K.: Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clin Chem Acta, 1978; 90: 37-43.
17. Lang, C.A., Naryshkin, S., Shneider, D.L., and Mills, S.B.: Low blood glutation level in healthy aging adults. J Lab Clin Med, 1992; 120: 720-5.
18. Menden, E.E., Balno, J.M., Murthy, L., and Petering, H.G.: Modification of a phenylene diamine oxidase method to permit non automated ceruloplasmin determination in batches rat serum or plasma microsamples. Anals Lett, 1979; 10: 197-204.
19. Diedrich, F., Renner, A., Rath, W., Kuhn, W., and Wieland, E.: Lipid hydroperoxides and free radical scavenging enzyme activities in preeclampsia and HELLP, Am J Obstet Gynecol, 2001; 185(1): 166-72.
20. Reinherd, G.: Shifts in the TH_1 /TH_2 Balance during human pregnancy correlate with apoptotic changes. Biochem Biophys Res Commun, 1998; 245: 933-8.
21. Fournel, S., Aguerre-Girr, M., Huc, X., Lenfant, F., Alam, A., Toubert, A., et al.: Cutting edge: soluble HLA-G1 triggers CD95/CD95 ligand-mediated apoptosis in activated CD8+ cells by interacting with CD8. J Immunol, 2000; 164(12): 6100-4.
22. Hsu, C.D., Harirah, H., Basherra, H., and Mor, G.: Serum soluble Fas levels in preeclampsia. Obstet Gynecol, 2001; 97(4): 530-2.
23. Daunter, B.: Eur J Obstet Gynecol Reprod Biol, 1992; 43: 81-95.
24. Chiu, P.M., Ngan, Y.S., Khoo, V.S., and Cheung, A.N.: Apoptotic activity in gestational trophoblastic disease correlate with clinical outcome. Histopathology, 2001; 38(3): 245-9.
25. Li, R., Wang, Z., Wu, R.: Study on decidual villous cell apoptosis and it is control gene in medical abortion. Zhonghua Fu Chan Ke Za Zhi, 1999; 34(5): 281-3.
26. Qumsiyeh, M.B., Kim, K.R., Ahmed, M.N., and Bradford, W.: Cytogenetics and mechanisms of spontaneous abortions: increased apoptosis and decreased cell proliferation in chromosomally abnormal villi. Cytogenet Cell Genet, 2000; 88(3-4): 230-5.
27. Shiraishi, H., Hayakawa, S., and Satoh, K.: Murin experimental abortion by IL2 adminstration is caused by activation of cytotoxic T-lymphocyte and placental apoptosis. J Clin Lab Immunol, 1996; 48(3): 93-108.
28. Fortunato, S.J., Menon, R., Bryant, C., Lombardi, S.J.: Programmed cell death (apoptosis) A possible pathway to metalloproteinase activation and fetal membrane degradation in premature rupture of membranes. Am J Obstet Gynecol, 2000; 182(6): 1468-76.