RESEARCH PAPER | Medical Science | Volume : 4 | Issue : 8 | August 2014 | ISSN - 2249-555X

Leukocytes Count and Oxidative Stress Markers in Blood and Follicular Fluid in Infertile Women Undergo Intracytoplasmic Sperm Injection (ICSI)

KEYWORDS: Oxidative stress, Malondialdehyde, Glutathione, Catalase enzyme,Leukocytes.

Sami R. Al-Katib	Basima Sh. AlGhazali	Ban J. Edan
Department of Physiology-Collage of Medicine-University of Kufa, Iraq	Department of Obs. & Gyn.-Collage of Medicine-University of Kufa, Iraq	Department of Physiology-Collage of Medicine-Babylonian University,Iraq

ABSTRACT *Activated leukocytes in both of monocytes and granulocytes, generate excess reactive oxygen speciese (ROS) resulting in oxidative stress which may affect oocyte quality and ultimately affect ART success. The objectives of this study were to evaluate the association between the different leukocyte types and Malondialdehyde (MDA) levels as a free radical marker; reduced glutathione (GSH) and Catalase enzyme as antioxidant in patients undergo intracytoplasmic sperm injection (ICSI).To carry this aim a total seventy-five infertile women aged between 22-45 years(31.43 ± 5.38 years), referred to the fertility clinic in Al-Sadder teaching hospital and undergone intracytoplasmic sperm injection throughout period from March 2013 to January 2014 were included in this study. MDA, GSH and CAT were measured by spectrophotometer. The results of this study showed that there was significant positive correlation between S.MDA level and WBC count, lymphocyte count and neutrophil count.There was insignificant negative correlation between WBC count, lymphocyte count , neutrophil count and neutrophil / lymphocyte ratio with FF.GSH .While S.CAThad significant negative correlation with Eosinophil count at p<0.05.*

Introduction

Innate immunity is being detected as powerful force for the regulation of tissue disintegration and integrity through sterile inflammation (Medzhitov, 2010). The ovary is a site with controlled tissue damage and restore, which have been judged as footmarks of innate immunity (Spanel-Borowski, 2010).Segmented leukocytes heavily populate the follicle wall being neutrophils or eosinophilsclearly in dependence of the species and of the early or late ovulation phase, in human follicles in the transition to a corpus luteum (CL), Eosinophil leukocytes pool relate to 90%. Eosinophils are concerned in immunoregulation, angiogenesis and tissue repair(Blanchard and Rothenberg, 2009). Activation ofleukocyte produces an inflammatory response, which is associated with increased production of ROS (Holtheet al.,2004).The follicular fluid contains leukocytes, macrophages, and cytokines, all of which are known of ROS. Activated leukocytes, both monocytes and granulocytes, generate excess reactive oxygen specieses resulting in oxidative stress which may influence oocyte quality and ultimatelyeffectof assisted reproductive techniques (ART) success (Levente, 2012). ROS may play a role in the regulation of the expression of genes encoding some immunoregulators, cytokines, and cell adhesion molecules which are implicated in the pathogenesis of some diseases like endometriosis (Sharma etal.,2013). An increase in the manufacture of ROS in endometriosis is attributed to the activation of the immune system.Of interest, catalase activity as antioxidant-defense system is amplified in the follicular fluid of women treated Cytokine granulosa cell cultures (Bausenweinet al., 2010). Thus, Catalase activity could become a parameter of oxidative stress in assisted reproductive treatment. Hence, the objectives of this study were to evaluate the association between the different leukocyte types and Malondialdehyde (MDA) levels as a free radical marker, reduced glutathione (GSH) and Catalase enzyme as antioxidant in patients undergo intracytoplasmic sperm injection (ICSI).

MATERIALS AND METHODS

Study Population

A total seventy-five infertile women aged between 22-45 years (31.43 ± 5.38 years), referred to the fertility clinic in Al-Sadder teaching hospital and undergone intracytoplasmic sperm injection throughout period from March 2013 to January 2014. Measurement of MDA was based on the calorimetric reaction with thiobarbituric acid (TBA) to form pink color product,which could be measured by spectrophotometer(Lunec,1990).Determination of GSH depends on the action of sulfhydryl groups(Boyer,2000). Sulfhydryl group of GSH could reduce disulfide chromogen of 5,5´-Dithiobis 2.nitrobenzoic acid (DTNB) and change it to an intensely yellow compound which could measure its absorbance directly by spectrophotometer at 412 nm and it was directly proportional to the GSH concentration (Burtis and Ashwood,1999). Catalase activity was determined by the decrease in absorbance due to H2O2conception (Abi,1974).Total WBC and differential white blood cell count were measured with an auto analyzer (Germany).Statistical analysis was performed in this study using SPSS (Statistical Package for Social Science; Version 17) program. One sample t-test was used to estimate mean and stander deviation. Results are reported as (mean±SD). Pearson's correlation analysis was used for correlation. P<0.05 was considered statistically significant (Daniel,1999).

Results

The hematological tests of infertile women who undergo ICSI with reference range are. The mean± SD of WBC, Neutrophils and lymphocytes are 8.106.22 ± 2.242.86, 5.166.32 ± 1.638.85 and 2215.32 ± 525.39 respectively. All tests of infertile women had located within reference range (Table 1).

The oxidative stress markers in serum and follicular fluid of infertile women who undergo ICSI showed the mean± SD of S.MDA, F. MDA, S.GSH, F.GSH .S.CAT and F.CAT are 3.13 ± 1.96, 3.37 ± 1.61, 27.29 ± 13.50, 29.08 ± 8.68, 1.40 ±.85 and 1.81 ±1.11 respectively (Table 2).

Volume : 4 | Issue : 8 | August 2014 | ISSN - 2249-555X

Table 1: Hematological tests of infertile women who undergo ICSI

Test	All Patients	Reference value
WBC cell/mm^3	8,106.22 ± 2,242.86	4000 -11000
Neutrophil	5,166.32 ± 1,638.85	50-60% WBC
Eosinophil	215.64 ± 166.06	1-4% WBC
Basophil	57.89 ± 39.86	0.5-2% WBC
Monocytes	591.11 ± 210.66	2-9% WBC
Lymphocyte	2215.32 ± 525.39	20-40% WBC
Neutrophil / lymphocyte ratio	2.39 ± 0.77	-
Platelets /mm^3	257.67 ± 55.04	200,000 – 400,000
Hemoglobin gm/dl	12.34 ± 1.14	11.5 – 16.5
RBC count/mm^3	(4.64 ± 0.49)*10^6	(4.7 ± 0.30) *10^6

Table (2): Mean ±SD of Oxidative Stress Markers in serum and follicular fluid of infertile women

Oxidative Stress Markers	(Mean ± SD)
S.MDA µM	3.1344±1.96038
F.MDA µM	3.3708±1.61033
S.GSH µM	27.2897±13.50466
F.GSH µM	29.0816±8.68015
S.CAT U/ML	1.4025±.84710
F.CAT U/ML	1.8136±1.10842

Relation between Hematological Tests and Oxidative Stress

Hematological Testsand MDA

There was significant positive correlation between S.MDA level and WBC count, lymphocyte count, neutrophil count and neutrophil/lymphocyte ratio ($p<0.05$) and insignificant positive correlation between FF.MDA level with WBC count, lymphocyte count, neutrophil count and neutrophil / lymphocyte ratio ($p>0.05$)(Table 3).

Table (3):Correlation between MDA and white blood cells

		WBC	N	B	E	M	L	N/L ratio
S.MDA	r	0.332*	0.421*	0.103	0.214	0.188	0.346*	0.015
	p	S	S	NS	NS	NS	S	NS
FF.MDA	r	0.043	0.105	0.001	0.191	0.345	0.077	0.131
	p	NS	NS	NS	NS	NS	NS	NS

***S Correlation is significant at the 0.05 level (2-tailed), NS: No significant differences at P<0.05, r correlation coefficient**

Hematological Tests and GSH

There was insignificant negative correlation between WBC count, lymphocyte count, neutrophil count and neutrophil/ lymphocyte ratio with FF.GSH ($p>0.05$) (Table 4).

Table (4):Correlation between GSH and white blood cells

		WBC	N	B	E	M	L	N/L ratio
S.GSH	r	0.005	0.164	0.409	0.037-	0.415	0.193	0.010-
	P	NS	NS	NS	NS	NS	NS	NS
FF.GSH	r	0.195	0.235	0.024	0.327	0.242	0.156	0.043
	P	NS	NS	NS	NS	NS	NS	NS

r correlation coefficient, NS: No significant differences at P<0.05

Hematological Tests and CAT

There was significant negative correlation between Eosinophil count and S.CAT ($p<0.05$) as shown in table (5). Also, there was negative but insignificant correlation between WBC count, lymphocyte count, neutrophil count and neutrophil / lymphocyte ratio with S.CAT, FF.CAT level ($p>0.05$) (Table 5).

Table (5):Correlation between CAT and white blood cells

		WBC	N	B	E	M	L	N/L ratio
S.CAT	r	0.206	0.387	0.279	0.548*	0.133	0.242	0.120
	P	NS	NS	NS	S	NS	NS	NS
FF.CAT	r	0.152	0.454	0.180	0.102	0.188	.008	0.336
	P	NS	NS	NS	NS	NS	NS	NS

***S Correlation is significant at the 0.05 level (2-tailed), NS: No significant differences at P<0.05, r correlation coefficient**

Discussion

The hematological tests of infertile women who undergo ICSI with reference range. All tests of infertile women had located within reference range.This result disagreed with previous study that found that the total number of T lymphocytes increased significantly during superovulation also they found that stress was associated with elevated amounts of activated T cells (Gallinelli et al., 2001).There was significant positive correlation between S.MDA level and WBC count, lymphocytes, neutrophils count and neutrophil/ lymphocyte ratio ($p<0.05$) and insignificant positive correlation between FF.MDA level with WBC count, lymphocytes, neutrophils count and neutrophil/lymphocyte ratio ($p>0.05$).The correlation of the free radicals with neutrophil counts was significant relative to the total WBC counts (although the difference in the correlation of the MDA with the neutrophil or WBC counts was not very high), and this suggests that neutrophils may be similarly or slightly but more concerned in the oxidative stress (OS) status, as assessed by this test, in comparison to the overall WBCs, this result agreed with pervious study by Kotani and Sakane (2012). This is noteworthy because of the specific roles of neutrophils on the oxidative milieu in bloodstream and different tissues, including the vasculature. This is also useful as the basic information in considering the link between inflammation and oxidative stress in various pathophysiologies using the free radicals test, a recently established marker to easily assess the oxidative burden in clinical settings (Kotaniand Taniguchi, 2012). Although, the biologic mechanism(s) causal the correlation between neutrophils and free radicals remain unclear, there are possible explanations. Neutrophils directly reflect the relevant pathophysiology as a WBC subpopulation in comparison to the total WBCs (Kotani andSakane,2012). Neutrophils might secrete different types of inflammatory chemokines and cytokines (eg, interluekin-6)(Kasamaet al.,2005) and the inflammation triggered by these molecules might both induce an oxidative stress response and further inflammation via cell dysfunction in systemic organs(Kotaniand Taniguchi, 2012). Neutrophils-released mediators, such as leukotriene B4 that might cause Oxidative stress also activates the inflammatory pathway, however, this cycle of oxidative stress and inflammation in relation to neutrophils may partly explain the correlation between neutrophils and free radicals levels (Kotani andSakane,2012).The relationship of WBC and its subpopulations with oxidative stress-related markers has not been thoroughly examined; for instance, a significant positive relationship between sputum 8-isoprostane and

Volume : 4 | Issue : 8 | August 2014 | ISSN - 2249-555X

neutrophil counts (Kinnulaet al., 2007) as well as between blood 3-chlorotyrosine and WBC counts(Chenget al., 2008) has been reported.

There was an independent, significant, and positive correlation between neutrophil counts and free radicals levels in asymptomatic female subjects (Kotaniand Taniguchi, 2012). Oxidative stress, caused some hematological effects in the female Wister rat, these results are also steady with corresponding increase in the percentages of neutrophils and eosinophils especially in the OS induced group; all these have manifested in the high number of immature follicles in the OS induced group (Shugabaet al.,2012). Another study found that constant oxidative stress causes an increase of proinflammatory mechanisms provide a potent feedback loop for sustained oxidant stress(Ivailoet al., 2012) .Leukocytes and other phagocyte tear down bacteria, parasites and virus-infected cells with NO, O_2, H_2O_2, and OCl, those are potent oxidants and protect humans from infection. However, they cause oxidative damage and mutation to DNA(Uttaraet al., 2009).

Damage induced by ROS occurs through the modulation of cytokine expression and pro-inflammatory substrates via activation of redox-sensitive transcription factors (Cindrova-Davies et al.,2007).Neutrophil modulation is a central source of ROS, which cause cell damage especially to endothelial cell (Kolluruet al.,2012).ROS may play a part in the regulation of the expression of genes encoding some immunoregulators, cytokines, and cell adhesion molecules which are involved in the pathogenesis of endometriosis (Sharmaet al., 2013).

There was insignificant negative correlation between WBC count, lymphocyte count, neutrophil count and neutrophil / lymphocyte ratio with FF.GSH ($p>0.05$).The non-antioxidants prevent damage induced by ROS through the modulation of cytokine expression and pro-inflammatory substrates by inhibiting the activation of NF-kappa B (Cindrova-Davieset al.,2007).There was significant negative correlation between Eosinophil count and S.CAT ($p<0.05$). Also, there was negative but insignificant correlation between WBC count, lymphocyte count, neutrophil count and neutrophil/lymphocyte ratio with S.CAT, FF.CAT and C.CAT level ($p>0.05$). Reducing antioxidant enzymes related with are an increase in prostaglandin (PG) F2-alpha or macrophages (Agarwal et al.,2012).Heme oxygenase-1 is an antioxidant enzyme that has anti-inflammatory and cytoprotective properties. Hypoxia stimulates the expression of HO-1, and is used to discover increased OS (Li et al, 2005 and Agarwalet al.,2012).

REFERENCE

Abi,H.(1974). Method of enzymatic analysis. New York. Academicpress; 2: 674-684. | Agarwal A., Aponte-Mellado A., Premkumar B., Shaman A.,1 and Gupta S. (2012) . The effects of oxidative stress on female reproduction: a review. ReprodBiolEndocrinol.; 10: 49. | Blanchard, C. and Rothenberg, M.E. (2009).Biology of the eosinophil.AdvImmunol.; 101, 81–121. | Boyer R. Modern Experimental Biochemistry.3rd ed. San Francisco, Addisoi Wesley Longman (editor), (2000). | Burtis, C.A. and Ashwood, E.R. (1999).Textbook of Clinical Chemistry, 3rd ed., Philadelphia, W.B.Saunders Co. | Cheng, M.; Chen, C.; Gu, P.; Ho, H. and Chiu, D.(2008). Elevated levels of myeloperoxidase, white blood cell count and 3-chlorotyrosine in Taiwanese patients with acute myocardial infarction. ClinBiochem; 41: 554-60. | Cindrova-Davies, T.;Yung, H.; Johns, J.; Spasic-Boskovic, O.; Korolchuk, S.; Jauniaux, E.; Burton, G. and Charnock-Jones, D. (2007). Oxidative stress, gene expression, and protein changes induced in the human placenta during labor. Am J Pathol; 171:1168-1179. | Daniel,W.W.(1999).Probability and t distribution biostatistics:A foundation for analysis in health science.7th ed.83-123.John willey and Sons,INC-USA. | Gallinelli, A., Roncaglia, R., Matteo, M., Ciaccio, I., Volpe, A. and Facchinetti, F. (2001). Immunological changes and stress are associated with different implantation rates in patients undergoing in vitro fertilization-embryo transfer. FertilSteril; 76, 85–91. | Holthe, M. ; Staff, A. ; Berge, L. and Lyberg, T. (2004). Leukocyte adhesion molecules and reactive oxygen species in preeclampsia.Obstet Gynecol.;103:913–922. | Ivailo, V., Julieta, D., Krassimira, T., Soren, H. and Maria, D. (2012). Ovarian Biomarkers in Infertility, Trends in Immunolabelled and Related Techniques, Dr. EltaybAbuelze in (Ed.) | Kasama, T.; Miwa, Y.; Isozaki, T.; Odai, T.; Adachi, M. and Kunkel,S. (2005).Neutrophil-derived cytokines: Potential therapeutic targets in inflammation. Curr Drug Targets Inflamm Allergy; 4:273-9. | Kinnula, V.; Ilumets, H.; Myllärniemi, M.; Sovijärvi, A. and Rytilä, P. (2007).8-Isoprostane as a marker of oxidative stress in nonsymptomatic cigarette smokers and COPD.EurRespir J;29:51-5. | Kolluru,G.; Bir, S. and Kevil,G.(2012). Endothelial Dysfunction and Diabetes: Effects on Angiogenesis, Vascular Remodeling, and Wound Healing. International Journal of Vascular Medicine;30. | Kotani, K. and Sakane, N. (2012). White Blood Cells, Neutrophils, and Reactive Oxygen Metabolites among Asymptomatic Subjects.International Journal of Preventive Medicine; 3(6). | Kotani, K. and Taniguchi, N. (2012).Correlation between high sensitivity C-reactive protein and reactive oxygen metabolites during a one-year period among asymptomatic subjects. J Clin Med Res; 4: 52-5. | Levente, L. (2012). The Role of Oxidative Stress in Female Reproduction and Pregnancy, Oxidative Stress and Diseases, Dr. VolodymyrLushchak (Ed.), http://www.intechopen.com/books/oxidative-stress-and-diseases/the-role-of-oxidative-stress-in-femalereproduction-and-pregnancy. | Li, H.; Gu, B.; Zhang, Y.; Lewis, D. and Wang, Y. (2005).Hypoxia-induced increase in soluble Flt-1 production correlates with enhanced oxidative stress in trophoblast cells from the human placenta. Placenta p 26:210-217. | Lunec, J. (1990). Review Article, Ann Clin. Biochem; 27: 173. | Medzhitov, R. (2010). Innate immunity. Nat Immunol; 11:551–553. | Sharma, K.; Reynolds, N.; Rakhit M. and Agarwal, A. (2013).Methods for Detection of ROS in the Female Reproductive System. Studies on Women's Health, Oxidative Stress in Applied Basic Research and Clinical Practice.33-60. | Shugaba, A.I.; Ojo, S. A.; Asala, A.S.; Rabiu, A.M.; Uzokwe, C.B. and Hambolu, J.O.(2012). White blood cells` response of female wistar rats following induced physical and oxidative stress. Global Advanced Research Journal of Medicine and Medical Sciences; 1(8): 203-207. | Spanel-Borowski, K. (2010). Footmarks of innate immunity and cytokeratin-positive cells as potential dendritic cells. | Spanel-Borowski, K. (2011). Ovulation as danger signaling event of innate immunity. Molecular and Cellular Endocrinology;333(1):1-24. | Uttara, B.; Singh, A.; Zamboni, P. and Mahajan, R. (2009).Oxidative Stress and Neurodegenerative Diseases: A Review of Upstream and Downstream Antioxidant Therapeutic Options. CurrNeuropharmacol.; 7(1): 65–74. |