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EVALUATION OF DNA OXIDATIVE DAMAGE BY 8-OHDG BIOMARKER DURING SPERM ACTIVATION WITH PLATELET-RICH PLASMA AND GLASS WOOL FILTRATION TECHNIQUES

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ABSTRACT

Background: Seminal oxidative stress (OS) is known as one of the important factors of male infertility through pathogenesis of sperm dysfunction and DNA damage. Oxidative DNA damage can lead to multiple forms of mutations as a result of structural alterations of bases as well as helix distortions and the production of single and double-strand DNA breaks. The most common product of oxidative DNA damage is oxidation of guanine to form 8-hydroxyguanine (8-oxo-G) or 8-hydroxy-2' -deoxyguanosine (8-OHdG). If left unrepaired this base will pair with adenine instead of cytosine. So accumulation of 8-OHdG in DNA may result in various pathologies and the significantly altered level of base damage analyzed by 8-OHdG is a potentially early diagnostic biomarker for such pathologies. **Objective:** Detection of oxidative DNA damage of the spermatozoa by measuring the 8-OHdG biomarker during sperm activation with Platelet-Rich Plasma(PRP) and Glass Wool filtration(GWF) techniques. **Patients and Methods:** The study included 60 infertile men as (45Asthenozoospermic,15 Normozoospermic) which were involved, their semen samples were analyzed and then activated with PRP and filtered with GWF, during their attendance to the infertility clinic of the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, the 8-OHdG biomarker was measured for each semen sample, three(3) readings were obtained as: before activation, after Glass Wool Filtration and after Platelet -Rich Plasma with Glass Wool Filtration sperm activation techniques**. Conclusion:** After comparison between the two groups of infertile men and measuring 8-OHdG biomarker, the latter has been found to be less concentration in normozoospermic than asthenozoospermia which is sensitive indicator for DNA damage, the concentration of 8-OHdG was also less following activation with Platelet -Rich Plasma and Glass Wool Filtration, than its concentration before activation.

KEYWORDS: Glass Wool Filtration(GWF), Platelet –Rich Plasma (PRP),8-Hydroxydeoxyguanosine(8-OHdG), Reactive oxygen species(ROS).

INTRODUCTION

The 8-Hydroxydeoxyguanosine (8-OHdG) was reported as a potential marker of age related damage accumulation in several studies[1][2], quantization of 8-OHdG has been performed by several immunological techniques to assay this marker such as (Enzyme-linked immunosorbent assay (ELISA) and immunofluorescence) each method has its specific advantages and disadvantage, and their reliable use of this biological alteration as a diagnostic biomarker for DNA damage in human disorders.[3] Measurement of 8-hydroxydeoxyguanosine (8-OHdG) offers a specific and quantitative biomarker on the extent of oxidative DNA damage caused by reactive oxygen species(ROS) in human sperm. The close correlation of 8-OHdG level with male infertility, function and sperm parameters indicates the potential diagnostic value of this technique in clinical applications.[4] The presence and accumulation of ROS can cause oxidative damage to the sperm DNA,which in turn may cause DNA related pathologies, thus 8-OHdG was reported as a potential biomarker to detect age related damage accumulation in several studies[1][2][5] it was found that the level of sperm 8-OHdG in infertile men was significantly higher than that in healthy subjects.[6]

PATIENTS AND METHODS

In this study that included 60 patients were divided into two groups of infertile men as 45 Asthenozoospermia and 15 Normozoospermia, for each man the semen sample was divided for 3 portions :1st portion as before activation 0.5 ml, 2nd portion 1 ml after activation by

Glass Wool Filtration(GWF),3rd portion 1 ml following PRP activation with GWF, for all specimens sperm parameters were assessed microscopically, then 8-OHdG kit was used and prepared by special principle including two plates with weal to spread 0.2ml from each specimen then the addition of 10µl of 8-OHdG antibody which is contained in the kit box, and the addition of standard solution then read the concentration by reader after washing of the specimen then read the concentration by ELISA technique after a series of preparation with 8-OHdG kit.

RESULTS

From comparison of the concentration of 8-OHdG it was found that its concentration is significantly higher in infertile Asthenozoospermic group, it was more than infertile Normozoospermic group 12.38±6.59 versus 8.97±3.06 respectively, post-activation regarding Normozoospermia group, there was a highly significant decrease in level of 8-OHdG following GWF and PRP activation in comparison with Glass Wool filtration alone (P=0.001). Regarding Asthenozoospermia group there was highly significant decrease of mean 8-OHdG following activation with GWF and PRP (P=0.001),but PRP resulted in more significant decrease than the GWF alone (P=0.001).Table 1 and Figure 1,2 can summarize and illustrate the results above.

Table 1:The level of 8-OHdG in infertile men enrolled in the current study before and after activation using Glass Wool and PRP as categorized into asthenozoospermic and normozoospermic groups.

Characteristic	Total *n* = 60	Normozoospermia *n* = 15	Asthenozoospermia *n* = 45	*p*
8-OHdG at before activation				
Mean ±SD	11.53 ±6.07 A	8.97 ±3.06 A	12.38 ±6.59 A	0.059 I NS
Range	0 -41.04	0 -13.58	6.03 -41.04	
8-OHdG after activation by GWF				
Mean ±SD	8.41 ±1.31 B	8.53 ±1.47 A	8.37 ±1.27 B	0.673 I NS
Range	4.6 -11.88	5.41 -11.88	4.6 -11.09	
8-OHdG after activation by GWF and PRP				
Mean ±SD	7.67 ±2.20 C	7.17 ±2.66 C	7.83 ±2.03 C	0.319 I NS
Range	0.93 -10.81	0.93 -9.61	0.93 -10.81	
p	0.001 Ph HS	0.001 Ph HS	0.001 Ph HS	

n: number of cases; **SD**: standard deviation; **PRP**:; **I**: Independent samples *t*-test; **Ph**: Phillais multiple repetition test; **NS**: not significant at $p > 0.05$; **HS**: highly significant at $p \leq 0.01$; capital letters (A and B) were used to indicate level of significance following conductance of post **LSD** multiple comparison test so that similar letters indicate no significant difference at $p > 0.05$ and different letters indicate significant difference at $p \leq 0.05$.

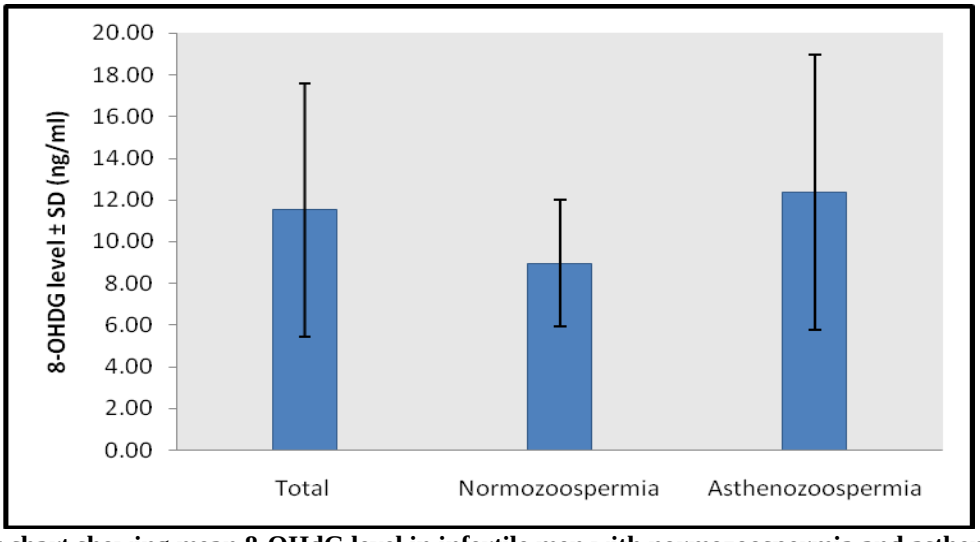

Figure 1: Bar chart showing mean 8-OHdG level in infertile men with normozoospermia and asthenozoospermia before activation.

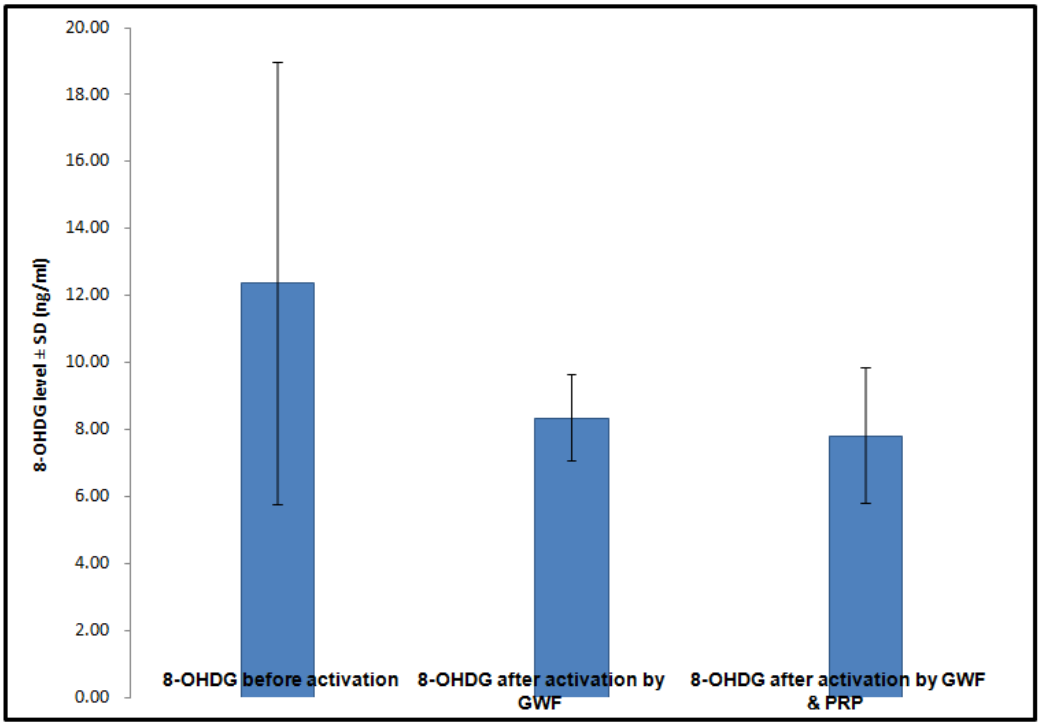

Figure 2: Bar chart showing mean 8-OHdG level in infertile men with asthenozoospermia before activation, after activation by glass wool and after activation by GWF and PRP.

DISCUSSION

This is the first study in Iraq that measured and compared the level of 8-OHdG in seminal fluid pre and post activation with PRP and Glass Wool Filtration techniques, however accurate and reproducible quantities of 8-OHdG damage requires immune effort and a particularly is a challenging issue in different types of cells, The validation of newly developed diagnostic biomarker such as 8-OHdG comprises the search for the most appropriate assay technology, recently immunological assay (ELISA,IF,AKLIDES) are used, one paper had pointed to this issue and focused on the reliable use of this biomarker for DNA damage in human.[3]

This research was designed to evaluate this oxidative damage by measuring the level of this biomarker pre-activation and post activation with Glass Wool and PRP methods in two groups of patients: Normozoospermic and asthenozoospermic Ones.

Oxidative DNA damage refers to various types of functional and or/ structural changes due to the reaction of ROS with DNA.[7,8].The formation of 8-Hydroxydeoxyguanosine (8-OHDG) is widely considered as key biomarker of oxidative DNA damage and plays an important role in the pathogenesis of many diseases[9], Some studies have shown that the sperm 8-OHDG level is closely associated with the presence of antioxidants in semen[7][8][10] Many other Articles and /or Researches have pointed to this association.[111,12,13]

The result of this study agree with the findings of some experimental studies that had shown the role of PRP in sperm activation which is superior than the Glass wool in upgrading the percentage of progressively motile sperm.[14] There is no previous studies / or articles about this issue in human, other animal experimental studies has pointed to the role of PRP in obtaining better sperm quality in term of progressive motility.

Platelet activating factor (PAF) also plays important role in reproduction, PAF content in squirrel monkey sperm is significantly higher during the breeding season than the non -breeding season, PAF content in human sperm has a positive correlation with seminal parameters and pregnancy outcomes.[15]

So far, according to the results obtained from the current study and data available in the published articles, it shows that both PRP and Glass wool equally effective in eliminating leukocytes and minimizing the effect of agglutinated sperms, in the other hand PRP is superior to Glass wool in improving sperm quality and motility percentage and together are recommended as an effective and efficient method in improving semen quality during assisted reproductive techniques for infertile couples.

CONCLUSION

After comparison between the two groups of infertile men and measuring 8-OHdG biomarker, the latter has been found to be less concentration in normozoospermic than asthenozoospermia which is sensitive indicator for DNA damage in various body tissues and in this study

8-OHdG was measured in seminal fluid by ELISA Technique, the concentration of 8-OHdG was also less following activation with Platelet -Rich Plasma and Glass Wool Filtration, than its concentration before activation.

REFERENCES

1. Gan W, Nie B, Shi F, Xu XM, Qian JC, Takagi Y.,2012. Age-dependent increases in the oxidative damage of DNA, RNA, and their metabolites in normal and senescence-accelerated mice analyzed by LC-MS/MS: urinary 8-oxoguanosine as a novel biomarker of aging. Free Radic Biol Med, 52: 1700–7.
2. Gan, W., Cai, J., Hu, G., Chen, L., Sekiguchi, M., Shi, F., Hayakawa, H., Nie, B., 2013. Age dependent accumulation of 8- Oxoguanine in the DNA and RNA in various rat tissues.Oxide Med Cell Longev, 303181.
3. Kemal S. Korkmaz, 2018. Cancer, Laboratory, Department of Bioengineering), University of Technology Cottbus-Senftenberg, Germany, PP1-8.
4. 4-Hang-Ming Shen, Choon-Nam ONG., 2000.Detection of oxidative DNA damage in Human sperm and its association with sperm function and male infertility, Biology and Medicine. Article, 28(4): 529-536.
5. Wei Gan, Xin-Le Liu, Ting Yu, Yuan-Gao Zou, Ting-Ting Li,Shuang Wang, Jin Deng, Lan-Lan Wang and Jian-Ping Cai., 2018. Urinary 8-oxo-7,8-dihydroguanosine as a Potential Biomarker of Aging. Frontiers in Aging Neuroscience, Article, 34(10): 1-8.
6. Shery T. Homa, Wayne Vessey, Ana Perez – Mirranda, Tripat Riyait and Ashock Agrawal., 2015. Reactive Oxygen Species (ROS) in human semen: determination of a reference range. Journal of Assisted Reproduction and Genetics, 32(5): 757–764.
7. Azher S. Hindal, Hayder A. L. Mossa, Muayad S. Abood,2018. Reactive Oxygen Species Levels in Seminal Plasma in a Sample of Iraqi Infertile Men using Advanced Stimulatory Method for Activation of Spermatozoa. International Journal of Medical Research & Health Sciences, 7(12): 51–55.
8. Watfaa A. Abduljabar, Hayder A. L. Mossa, Muayad S. Abood, 2021. Beneficial Role of Platelet –Rich Plasma (PRP)combined with Glass Wool Filtration for Best Recovery of Spermatozoa in Infertile Men. European Journal of Clinical Medicine, 2(5): 11-13.
9. Ames BN, Shigenaga MK, Hagen TM, 1993. Oxidants, antioxidants, and the degenerative disease and aging. Proc Natl Acad Sci USA, 90: 7915-7922.
10. Fraga CG, Motchnik PA, Wyrobek AJ, Rempel DM, Ames BN, 1996. Smoking and low antioxidant levels increase oxidative damage to sperm DNA. Mutat Res, 351: 199-20.
11. Kadhim AA, Mossa HAL, Abbood MS, 2017. A comparison of new sperm preparation technique by glass wool filtration combined with pentoxifylline with other techniques in asthenozoospermic men. International Journal of Advanced Research, 5(4): 1178–1182.
12. Saad S. Al-Dujaily, Hayder A. L. Mossa, Sabah N. Alwachi, 2015. Study of Calpain Activity, Sperm Penetration Assay in Unexplained Infertile Men in Relevance to Assisted Reproductive Technologies. World Journal of Pharmaceutical Research, 4(2): 100-117.
13. Hamza NA, Selman MO, Mossa HA, 2018. Comparison of Best Yield of in vitro Sperm activation Techniques with New technique ofCaffeine Combined with Density Gradient Centrifugation in Iraqi Patients. Journal of Pharmaceutical Sciences and Research, 10(1): 36-39.
14. Bader, R., Ibrahim, J. N., Moussa, M., Mourad, A., Azoury, J., Azoury, J., & Alaaeddine, N., 2020. In vitro effect of autologous platelet- rich plasma on H2O2- induced oxidative stress in human spermatozoa. Andrology, 8(1): 191-200.
15. Roudebush W.E, Purnell E.T, 2000. Platelet – activating factor content in human spermatozoa. Fertil Steril, 74; 257-260.