



JBAAR



SPBH

Relationship between Sperm DNA Fragmentation and Interleukin 17 in Patients with Leukocytospermia

Hayder L F AL-Msaid¹, Hydar Muhsin Khalfa², Ammar Adil Rashid³ and
Nawras Noori Mohammad Hussain⁴

1,2,3: Department of Biology, College of Sciences, University of Kufa, Najaf, Iraq.

4: Al -mustaqbal University. College of medical and health techniques and science, Department of Medical Laboratory Techniques, Iraq

Corresponding author email: haiderl.ligan@uokufa.edu.iq

DOI:10.21608/jbaar.2024.392991

Abstract:

Over the past few decades, fertility treatments have witnessed a remarkable development, inflammation in the sexual glands attached to some patients in addition to immune disorders in patients, which are among the main causes of delayed pregnancy. A hidden molecular cause of infertility is sperm DNA fragmentation which can be attributed to many cases of infertility. This study aims to correlate the relationship between sperm DNA fragmentation and interleukin 17A in patients who suffer from leukocytospermia. Samples were collected from patients with Leukocytospermia (n=30) and healthy individuals (n=18). The present study showed a significant increase in Interleukin 17A levels in Leukocytospermia patients compared with the control group (mean± Std. Error 356.5± 6.14 & 141.37± 1.32) also significant sperm DNA Fragmentation level was noticeably decreased in Leukocytospermia compare with control group (mean± Std. Error 30.85±1.17 & 79.16±3.24 Consecutive). The results show a negative correlation between the Interleukin 17A level and sperm DNA Fragmentation level in Leukocytospermia patients. The study concluded the inflammation of patients affects an increase in the percentage of abnormal sperm chromatin that was assessed using the aniline blue dye. An increase in the level of pro-inflammatory such as interleukin 17 plays a critical role in the pathogenesis and inflammation, while a negative correlation between (Interleukin 17A level and the sperm DNA Fragmentation level in Leukocytospermia patients.

Keywords: Sperm DNA Fragmentation, IL17, Leukocytospermia.

INTRODUCTION

One of the cells specializing in fertilization is where it works to deliver the male genetic material to the goal required to induce pregnancy [1]. Research interest in DNA fragmentation in spermatozoa has taken place in the past two decades, where it has found many changes that have led to mutations that are not benign [2]. The fragmentation of DNA in sperm in the past two decades has begun scientific research in several universities and the focus has become important to

them and opened up new horizons [3]. The fear of these genetic changes in men led to infertility, the difficulty of treatment, or the impossibility of treating it [4]. It is known that gloss proliferation solves many problems, but in nuclear problems, some problems are difficult to solve, as this led to it becoming a problem this time. Several studies have proven that after ICSI the presence of nuclear damage affects the outcome of pregnancy [5,6]. In this case, nuclear studies in human infertility have become very important, and this has led to less

fragmentation of DNA [7,8]. For the dream of fatherhood to be fulfilled, research was confirmed to determine the things that would lead to DNA damage to DNA, and this led to the addition of much knowledge in this scientific field, as this produced a move away from several things, including drugs, and other factors that would affect the fragmentation DNA [9]. Damage events in DNA have been affected by many internal factors, including increasing free radicals, as well as apoptosis events, which are important for causing damage [10,11]. Some researchers believe that DNA damage is caused by the presence of immature sperms that increase ROS [12]. Finally, it is believed that the failure of the association between DNA nucleic acid molecules and the replacement of histone during the maturation process of chromatin led to apoptotic events in a significant way [13]. Urinary tract infection is one of the reasons that contribute to the infertility of men. When the number of white cells reaches 1-2 million / ml, the semen bleaching is considered average, while this bleaching is noticeable (Marked) when the number of these cells reaches (2-5) million / ml of semen and is accompanied by clusters of macrophages that Eating sperm [14]. One of the studies found that there is a relationship between bacterial, viral, and fungal infections and DNA properties as well as a decrease in physiological parameters and this may lead to a lack of sperm [15]. One of the roles IL-17 will play is its involvement in the inflammatory response, where this is with allergies, and this leads to the secretion of cytokines, chemokines, and other factors within the immune response [16,17]. The action of IL-17 with IL-22 together leads to an immune response against microbes by producing an anti-peptide, which is in the keratinocytes [18]. The immune response that leads to IL-17 responses should increase in neutrophils, and this condition occurs as airway remodeling [19]. In some diseases, including asthma, anti-tumor immunity, and some arthritis diseases, the work of IL-17 is very necessary to stimulate CD4 + T-cells called T helper 17 [20]. Some research that studies the role of IL 17

indicated the very important role of the local tissue disease in the IL 17 effect of the release of the pro-inflammatory cytokines and chemokines [21]. In addition, other research provided the IL 17 that stimulates some genes, which is responsible for the production of growth factors and some peptide against some microbes, also other researchers provided the IL 17 that stimulates neutrophil formation [22].

MATERIALS AND METHODS

Patient selection: serum and semen from infertile patients as well as the control group from the infertility center of Al-Sadr Teaching Hospital. The classification of infertility was characterized by microscopic examination. The average age of patients was (35.76±44) years. A total of 60 samples were collected. Interleukin 17 global company interleukin 17A (MBS163815) MYBIOSOURCE USA in Origin) examination was performed on 48 samples using an immunological method (Enzyme-Linked-Immuno-Sorbent- Assay) by using an ELISA reader (Huma Germany origin). All fluids were handled with care and used at room temperature to prevent any errors and follow the manufacturer's instructions. Sperm chromatin assessment was performed with aniline blue staining (Mohammad et al,2012). All examinations were carried out at the College of Science, Kufa University.

RESULTS

The results show a significant increase in Interleukin 17A in Leukocytospermia subjects compared to control subjects (mean± Std. Error 356.5± 6.14 & 141.37± 1.32) also significant sperm DNA Fragmentation level was decreased in Leukocytospermia in comparison with that of healthy individuals (mean± Std. Error 30.85±1.17 & 79.16±3.24 Consecutive). The results show a negative correlation between the interleukin 17A level and the sperm DNA Fragmentation level in Leukocytospermia patients. The result also shows apparent Semen and sperm parameters for Leukocytospermia, compared to that in the control group.

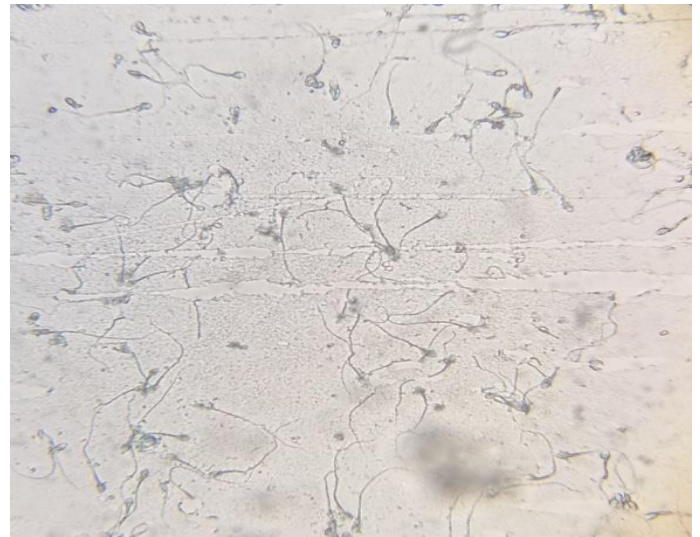
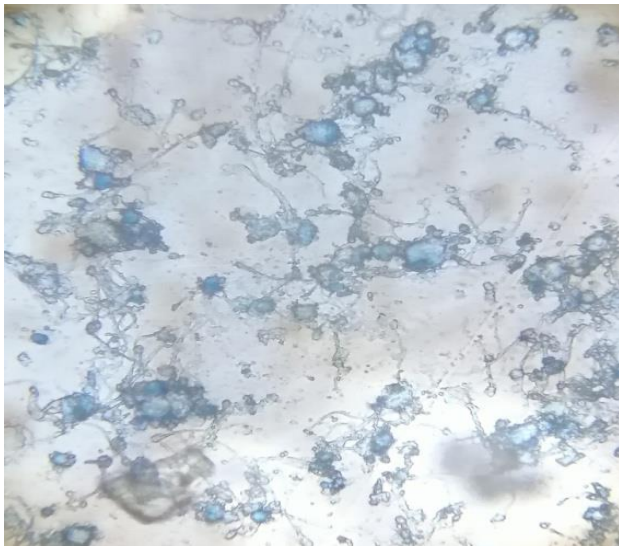


FIGURE 1. Sperm DNA fragmentation in patients with Leukocytospermia X40

FIGURE 2. Sperm DNA Fragmentation in control group X40

Table (1): Semen and sperm parameters for Leukocytospermia, compared to the control group

| Studied parameters | Leukocytospermia | control group |
|--------------------------|------------------|---------------|
| Sperm count million /ml | 32 ± 2.45* | 79.56± 5.15 |
| Sperm progressive motile | 28.42± 2.41* | 79.17±1.53 |
| Sperm normal morphology | 34.36± 2.29 * | 62.78± 1.77 |
| Viability sperm test | 33.28± 2.48* | 85.83± 0.83 |

The results inside the table represent mean± Std. Error,* Denotes significant (P <0.05)

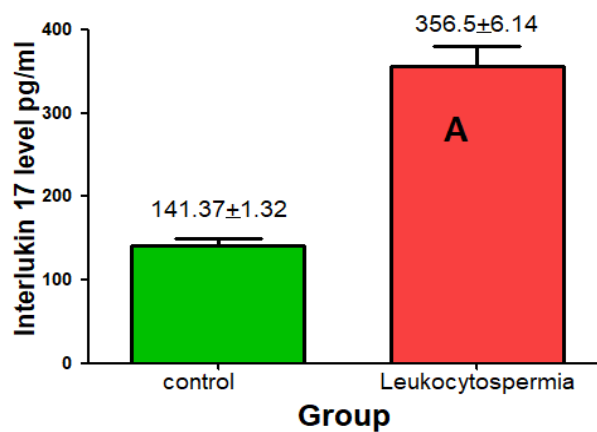


FIGURE 3. Comparison of Interleukin 17 level in the serum between Leukocytospermia compared with the control group and unexplained men infertility, The difference later indicates significant (p < 0.05)

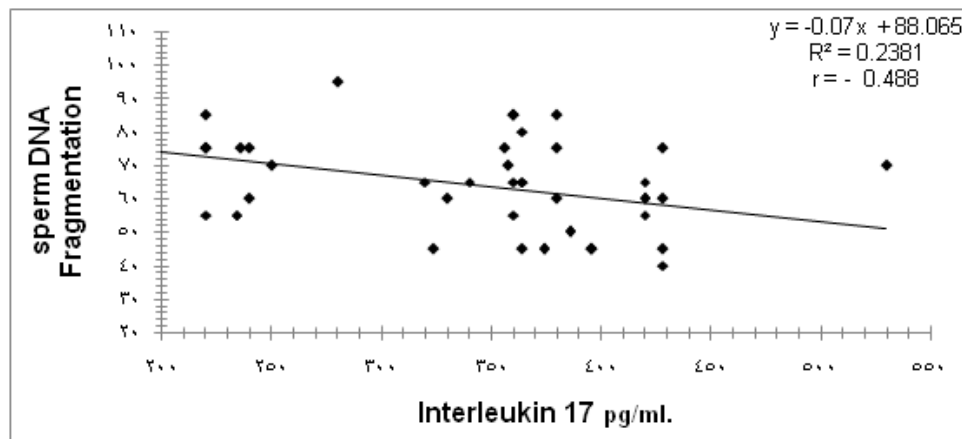


FIGURE 3. Correlation between Interleukin 17 with sperm DNA fragmentation in Leukocytospermia.

DISCUSSION

This study showed a significant increase ($p < 0.05$) in Interleukin 17 level in Leukocytospermia compared to that of a control group. The immune system plays a vital role in male infertility. Cytokines released during this stimulation are produced by various cells in response to pro-inflammatory responses. Interleukin 17A plays a critical role in the pathogenesis and inflammatory response which is secreted by T cell response to damaged tissues [23]. A study showed that patients with azoospermia measured interleukin (IL-17) levels in testicular biopsies of azoospermic patients using real-time PCR and immunohistochemistry. The study involved 60 males that show Sixty-two males and was divided into three groups, the first group included 25 patients whose semen deteriorated gradually reaching Azoospermia [24-27]. The second group included 25 patients diagnosed with obstructive azoospermia either congenital or acquired and the last group included 12 subjects with normal semen analysis who failed to provide a semen sample on the day of ICSI as a control group. IL-17 expression level showed elevation in infertile men compared with control [28,29].

The results of this study showed a significant decrease in sperm DNA fragmentation for patients

with Leukocytospermia compared with the control group, and the reason may be due to an imbalance in the process of mobilization of sperm chromatin and the process of programmed apoptosis of cells. Another possibility can be due to a high level of reactive oxygen species (ROS) caused by infection or diabetes mellites, which in turn leads to a condition of Oxidative stress and thus increased damage to chromatin or DNA of sperm [30-33].

CONCLUSION

Inflammation of patients leads to an increase in the percentage of abnormal sperm chromatin that was assessed using the aniline blue dye. An increase in the level of pro-inflammatory molecules such as interleukin 17 plays a critical role in the pathogenesis and inflammation, while a negative correlation between (Interleukin 17A level and the sperm DNA Fragmentation level in Leukocytospermia patients.

Conflict of interests: None

Funding: None

REFERENCES

1. Lee, S. E.; Jeong, S. K. and Lee, S. H. (2010). Protease and protease-activated receptor-2 signaling in the pathogenesis of atopic dermatitis. *Yonsei medical journal*, 51(6): 808-822.

2. Boerke, A., Dieleman, S. J., & Gadella, B. M. (2007). A possible role for sperm RNA in early embryo development. *Theriogenology*, 68, S147-S155.
3. Peter, C. J., Fischer, L. K., Kundakovic, M., Garg, P., Jakovcevski, M., Dincer, A., ...& Ratner, C. (2016). DNA methylation signatures of early childhood malnutrition associated with impairments in attention and cognition. *Biological psychiatry*, 80(10), 765-774.
4. Majzoub, A., Agarwal, A., Cho, C. L., & Esteves, S. C. (2017). Sperm DNA fragmentation testing: a cross sectional survey on current practices of fertility specialists. *Translational andrology and urology*, 6(Suppl 4), S710.
5. AL-Msaid, H., & AL-Sallami, A. (2018). Study of Catsper1 Protein Levels in Unexplained and Idiopathic Infertile Men. *International Journal of Pharmaceutical Quality Assurance*, 9(2), 195-198.
6. Comizzoli, P. (2015). Biobanking efforts and new advances in male fertility preservation for rare and endangered species. *Asian journal of andrology*, 17(4), 640.
7. Pukazhenth, B. S., & Wildt, D. E. (2003). Which reproductive technologies are most relevant to studying, managing and conserving wildlife?. *Reproduction, Fertility and Development*, 16(2), 33-46.
8. Eskarous, N., Hassab El-Nabi, S., Abd El Salam, M., Geba, K., Gamal El Din, S. Detection of DNA damage by SCD and Rate of Apoptosis DNA by Gel Electrophoresis among infertile males. *Journal of Bioscience and Applied Research*, 2022; 8(3): 181-189. doi: 10.21608/jbaar.2022.256197
9. Khalfa, H. M., Al-Msaid, H. L., & Alsahlane, R. (2019). Relationship between vitamin D3 levels and marital status: A random study in females suffering from bone disorders in Al-Najaf province. *Journal of Medical and Life Science*, 1(3), 65-70.
10. Souza, L. V., De Meneck, F., Fernandes, T., Oliveira, E. M., & Franco, M. D. C. (2020). Physical activity intervention improved the number and functionality of endothelial progenitor cells in low birth weight children. *Nutrition, Metabolism and Cardiovascular Diseases*, 30(1), 60-70.
11. Hole, P. S., Darley, R. L., & Tonks, A. (2011). Do reactive oxygen species play a role in myeloid leukemias?. *Blood, The Journal of the American Society of Hematology*, 117(22), 5816-5826.
12. Muratori, M., Marchiani, S., Tamburrino, L., & Baldi, E. (2019). Sperm DNA fragmentation: Mechanisms of origin. In *Genetic Damage in Human Spermatozoa* (pp. 75-85). Springer, Cham.
13. Bansal, A. K., & Kaushik, K. (2019). Role of Oxidative Stress, Reactive Oxygen Species & Antioxidants in Male Reproductive Functions. *Theriogenology Insight-An International Journal of Reproduction in all Animals*, 9(1), 35-45.
14. Muratori, M., Marchiani, S., Tamburrino, L., & Baldi, E. (2019). Sperm DNA fragmentation: Mechanisms of origin. In *Genetic Damage in Human Spermatozoa* (pp. 75-85). Springer, Cham.
15. Mao, W. (2019). Strategies for improving antitumor response in prostate cancer: BET Bromodomain inhibition and A2A Adenosine Receptor inhibition as methods of targeting prostate cancer (Doctoral dissertation, Johns Hopkins University).
16. Almsaid, H., & Khalfa, H. M. (2020). The effect of Ketogenic diet on vitamin D3 and testosterone hormone in patients with diabetes mellitus type 2. *Current Issues in Pharmacy and Medical Sciences*, 33(4), 202-205.
17. Oshiba, S., Harba, N., El Meleg, M., El Gammal, S., Hegazy, A., Abokhalil, N. Effects of COVID-19 vaccine on experimentally infected mice with *Schistosoma mansoni*. *Journal of Bioscience and Applied*

- Research*, 2023; 9(3): 138-160. doi: 10.21608/jbaar.2023.317937
18. Piao, C. H., Song, C. H., Lee, E. J., & Chai, O. H. (2020). Saikosaponin A ameliorates nasal inflammation by suppressing IL-6/ROR- γ t/STAT3/IL-17/NF- κ B pathway in OVA-induced allergic rhinitis. *Chemico-biological interactions*, 315, 108874.
 19. AL-Msaid Hayder L F & AL-SallamiAlaauldeen S M (2018). Study the level of cytokine in unexplained and idiopathic infertile men. *Journal of Pharmaceutical Sciences and Research.*; 10(4), 808-811.
 20. Ramakrishnan, R. K., Al Heialy, S., & Hamid, Q. (2019). Role of IL-17 in asthma pathogenesis and its implications for the clinic. *Expert review of respiratory medicine*, 13(11), 1057-1068.
 21. Koga, T., Ichinose, K., Kawakami, A., & Tsokos, G. C. (2019). The role of IL-17 in systemic lupus erythematosus and its potential as a therapeutic target. *Expert review of clinical immunology*, 15(6), 629-637.
 22. KhademAzarian, S., Jafarnezhad-Ansariha, F., Nazeri, S., Azizi, G., Aghazadeh, Z., Hosseinzadeh, E., & Mirshafiey, A. (2020). Effects of guluronic acid, as a new NSAID with immunomodulatory properties on IL-17, ROR γ t, IL-4 and GATA-3 gene expression in rheumatoid arthritis patients. *Immunopharmacology and Immunotoxicology*, 42(1), 22-27.
 23. Saini, C., Srivastava, R. K., Kumar, P., Ramesh, V., & Sharma, A. (2020). A distinct double positive IL-17A⁺/F⁺ T helper 17 cells induced inflammation leads to IL17 producing neutrophils in Type 1 reaction of leprosy patients. *Cytokine*, 126, 154873.
 24. Lea L uthje, F., Jensen, L. K., Elvang Jensen, H., & Skovgaard, K. (2020). The inflammatory response to bone infection A review based on animal models and human patients. *APMIS*.
 25. NA, M., MT, A., KM, S., & RS, M. (2020). IMPACT OF POLYCYCLIC AROMATIC HYDROCARBONS ON MALE REPRODUCTIVE HEALTH IN COAL TAR WORKERS. *Egyptian Journal of Occupational Medicine*, 44(1), 499-512.
 26. AL-Msaid, H. L., Waleed, H. A., & AL-Sallami, A. S. (2019). Relationship Between Hyperviscosity and Sex Hormone in Azoospermia and Oligozoospermia Patients Compares with The Control Group. *INTERNATIONAL JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH*, 10(04), 637-639.
 27. AL-Msaid, H. L., & Khalfa, H. M. (2019). Relationship between interleukin 17 & 6 in patients with varicocele compare with a control group. *Journal of Medical and Life Science*, 1(3), 71-74.
 28. Albideri, A. 2018. Histological and Cytoarchitectural Measurements of Human Epidermis in Different Anatomical Sites of Embryonic, Fetal and Neonatal Iraqi Subjects in Al-Hilla/Iraq Maternity Hospital. *Journal of Pharmaceutical Sciences and Research*, 10(4), 812-818.
 29. Albideri, A., & Jaffat, H. S. 2018. Cytological and Histological Study of Adult and Neonate Epidermis in Thick and Thin Skin of Various Anatomical Sites. *International Journal of Pharmaceutical Quality Assurance*, 9(2).
 30. khalfa, H. M., al-msaid, H. L., Abood, A. H., Naji, M. A., & Hussein, S. K. (2020, December). Cellular genetic expression of purinergic receptors in different organs of male rats injected with cyclophosphamide. In *AIP Conference Proceedings* (Vol. 2290, No. 1, p. 020033). AIP Publishing LLC.
 31. Yameny, A. Diabetes Mellitus Overview 2024. *Journal of Bioscience and Applied Research*, 2024; 10(3): 641-645. doi: 10.21608/jbaar.2024.382794
 32. AL-DUJAILI, A. N. G., HUSSAIN, M. K., & AL-FATLAWI, N. A. G. H. ASSESSMENT OF LEPTIN HORMONE AND VITAMIN C IN PATIENTS WITH TYPE 2 DIABETES

MELLITUS IN NAJAF GOVERNORATE.

Asian Jr. of Microbiol. Biotech. Env. Sc. 2016:

Vol. 18, No. (1): 67-73

33. SALEEM, K., ALHADRAWY, M. K. A.,
ABOOD, A. H., & HUSSAIN, H. M. K.
EVALUATION LEVELS OF

IMMUNOGLOBIN G AND LIPID PROFILE

IN PATIENTS INFECTED WITH HYATID

CYST IN AL-NAJAF GOVERNATE, IRAQ.

Asian Jr. of Microbiol. Biotech. Env. Sc. 2016:

Vol. 18, No. (1): 101-104