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Sperm DNA Fragmentation in Petroleum Workers

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ABSTRACT: The present study showed the sperm DNA fragmentation level in control group was 26.32±11.25, in office workers and in direct exposed workers were 2.35±14.54 and 46.73±16.28 respectively. The result revealed the significant difference between the control and the other two groups (P≤0.05) but no difference between the office workers and the direct exposed workers. Sperm DNA fragmentation (DFI) showed a correlation with the NQO1 (r) =-0.673.

KEYWORDS: Sperm, DFI, fragmentation, petroleum, DNA.

I. INTRODUCTION

Petroleum products are a fundamental part of our present day lives. It is almost difficult to stay away from exposure to hydrocarbons from petroleum products, whether it is from gasoline vapor at the pump, spilled crankcase oil on asphalt, solvents utilized at home or work, or pesticide applications that utilization petroleum products as transporters.

II. RELATED WORK

Spermatozoa with DNA damage are capable of fertilizing oocytes (Twigg *et al*., 1998; Gandini *et al*,2004). However, The development of the embryo can be influenced depending on the level of the DNA damage, and thus end in embryonic death (Tesarik *et al*., 2004). Gandini *et al*., (2004) found that the development of embryos formed with sperm damaged DNA can reach full term. Despite the apparent absence of a male or female infertility factor a relative high number of women fail to conceive. It is probably that many of these childless couples could have a genomic male contribution to infertility, which may include sperm DNA fragmentation, meiotic alterations, or sperm aneuploidy (Sakkas, and Alvarez,2010).Evidences showed that DNA fragmentation in sperm may cause conception failure , abortion ,malformation and genetic diseases . The male germ cells may be susceptible to environmental genotoxicants at different stages of spermatogenesis(Wu *et al*.,2015). Unrepaired DNA damage in spermatogonia may cause mutations during replication giving rise to mutated sperm. Independent DNA replication in association with recombination or DNA repair lead to the fixation of mutations (Olsen *et al*., 2005). The environmental agents can interact with the male genome and therefore the paternal exposure to these agents can influence on the offspring's future health and the fertility (Soubry *et al*., 201 4). Yauk *et al*.,(2007) quantified induced ESTR mutations to determine which stage of spermatogenesis that are susceptible to mutation and DNA modifications that caused from air pollution exposure. Kumar *et al*.,(2013) provided the first proof on the detrimental influence of occupational radiation exposure on functional, genetic and epigenetic integrity of sperm in health workers. However, further studies are required to confirm the potential detrimental effects of ionizing radiation in these subjects. Group of congenital malformations was discovered among the offspring of fathers previously working on a marine vessel in Norway(Mageroy, *et al*., 2006). Rubes *et al*., (2005) concluded that the exposure to intermittent air pollution may cause sperm DNA harm which in turn increase the male infertility, miscarriage, and other adverse reproductive results . Mandani *et al*.,(2013) found that The exposure to Catechol one of the benzene metabolites had an adverse effect on the sperm DNA. Kim *et al*.,(2010) compared between petrochemical workers with public servants in a non

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industrialized area and found that occupational exposure to petrochemical compounds decrease sperm motility and defect the sperm ultra structural arrangement. Exposures to other hydrocarbons, such as trichloroethylene, mineral oils, and polycyclic aromatic hydrocarbons present in cigarette smoke are strongly associated with childhood leukemia (Castro-Jimenez and Orozco-Vargas,2011).

III. MATERIALS and METHODS

Assessment of sperm DNA integrity in workers

Semen samples were collected from 30 men in sterile containers by masturbation after a period of 2 to 5 days sexual abstinence . A portion of each sample was analyzed for standard semen quality parameters according to the protocols of Cooper *et al*., (2010). The reminder was used to assess the DNA damage that caused from the exposure to petroleum products by Halo sperm assay . The participants were divided into three groups : control (unexposed), office workers and direct exposed workers.

The sperm chromatin dispersion (Halo) test

The Halo test is a simple and inexpensive assay . According to Fernandez *et al.*,(2003) by using the halosperm G2 kit.

IV. EXPERIMENTAL RESULTS

DNA fragmentation Indices (DFI)

Table(1,2,3) displayed the numbers of sperm with normal DNA which represented by the big and medium halos as seen in figure (6,7) and the sperm with damaged DNA represented by the small or without halo in addition to degraded ones figure (8-24). The sperm DNA fragmentation level in control group was 26.32±11.25 in which the higher level of DFI was 43.3% and the minimum was 11 % . The DFI levels in office workers and in direct exposed workers were 42.35±14.54 and 46.73±16.28 respectively Table (8). The maximum level was 65% in office workers and was 69% in the direct exposed workers while the lower level was 26.3% and 25 respectively. In both groups the ten samples showed an elevated rates of DFI. The result revealed the significant difference between the control and the other two groups ($P \leq 0.05$) but no difference between the office workers and the direct exposed workers. The correlation factor (r) = 0.815($P \leq 0.05$). The present study showed a negative correlation between sperm DNA fragmentation and sperm motility (r)= -0.883, $P \leq 0.05$ in which the motility levels decreased as the DFI levels increased (Table 10,11,12). In the other hand there was no correlation between sperm DNA damage and sperm morphology It can be concluded that normal sperm morphology does not necessarily refer to intact DNA but sometimes it contains damaged DNA as seen in figure (7) showed a sperm that has normal head but has DNA fragmentation .

Table (1) Correlation between DNA fragmentation and sperm parameters in Control

No.	DNA fra.	Mot.	Mor.
1	39.33	58	33
2	19.67	60	45
3	38.33	55	42
4	22.30	59	45
5	31.33	48	50
6	24.00	57	32
7	12.66	62	30
8	11.00	62	42
9	21.33	58	40
10	43.30	40	20
Mean ±SD	26.325±11.249	55.9±6.887	37.9±8.987

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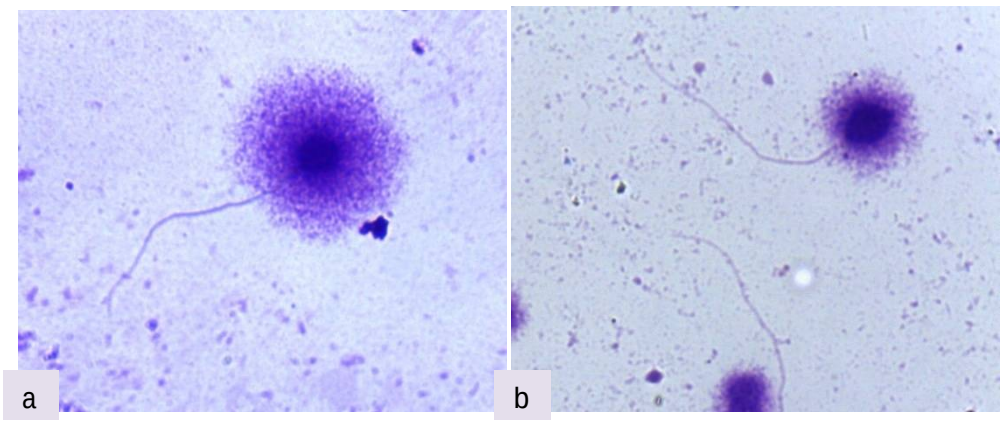
Table (2) Correlation between DNA fragmentation and sperm parameters in Office Workers

No.	DNA fra.	Mot.	Mor.
1	31.3	33	37
2	27.333	32	53
3	33.3	41	35
4	55.67	10	51
5	35.667	33	45
6	35	22	45
7	57.333	8	32
8	26.3	36	12
9	56.6	10	43
10	65	0	42
Mean ±SD	42.35±14.54	22.5±14.39	39.5±11.7

Table (3) Correlation between DNA fragmentation and sperm parameters in direct exposed

No.	DNA fra.	Mot.	Mor.
1	25	46	49
2	42.333	33	62
3	43.3	18	30
4	56	19	44
5	26.667	31	49
6	54.3	12	12
7	69	0	35
8	55.667	10	50
9	28	45	41
10	67	9	43
Mean ±SD	46.73±16. 28	22.30±15.73	41.50 ± 13.57



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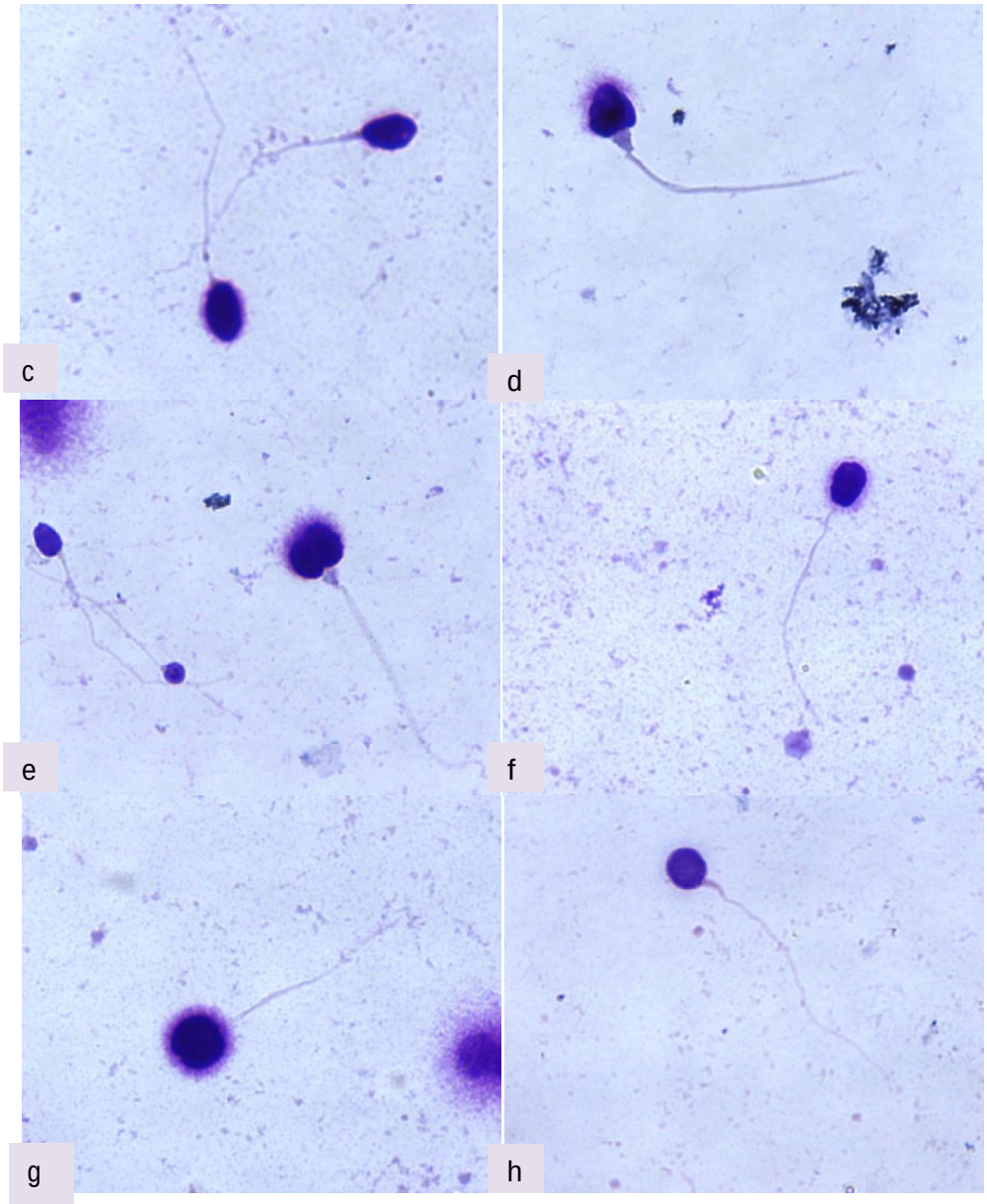

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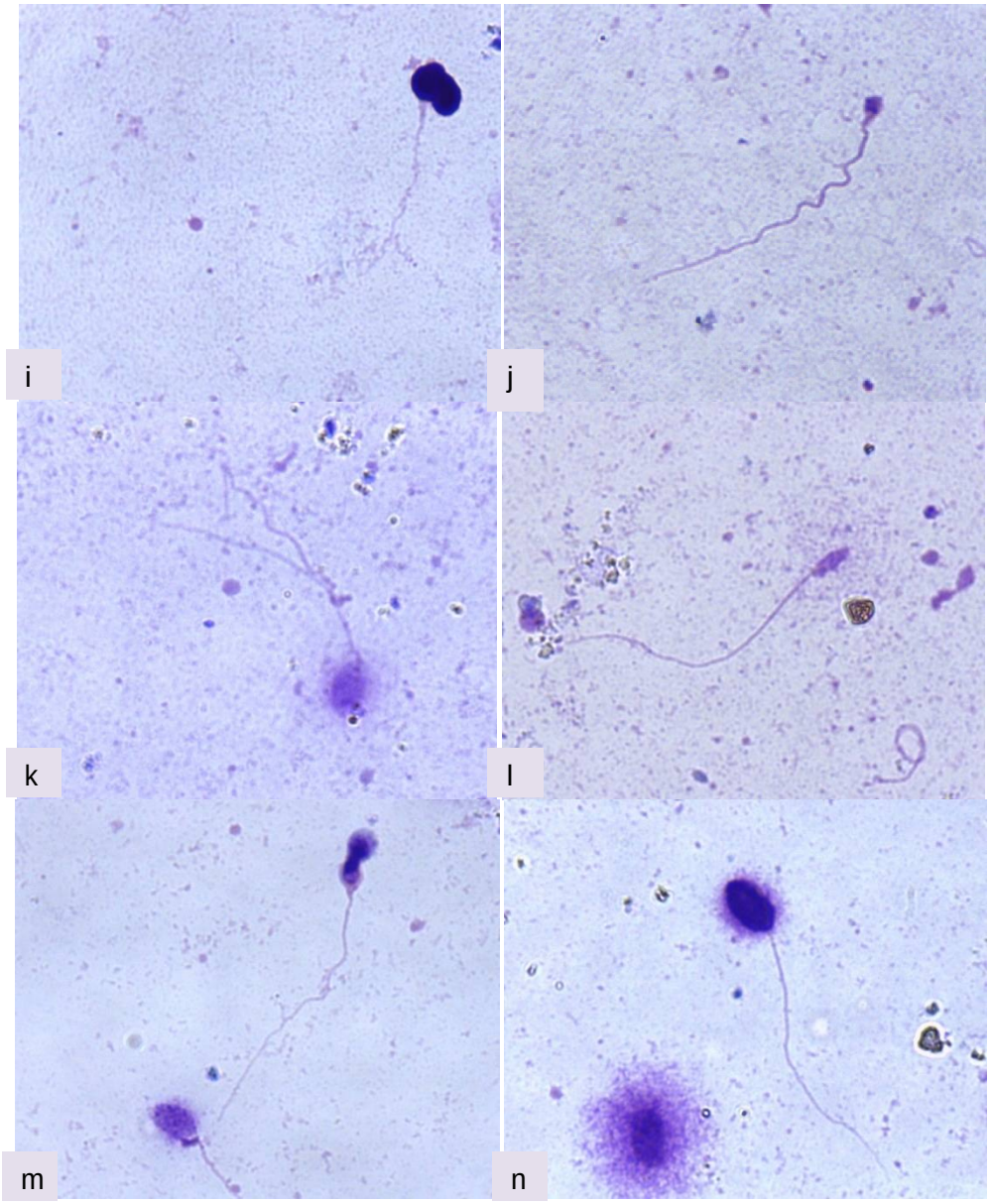

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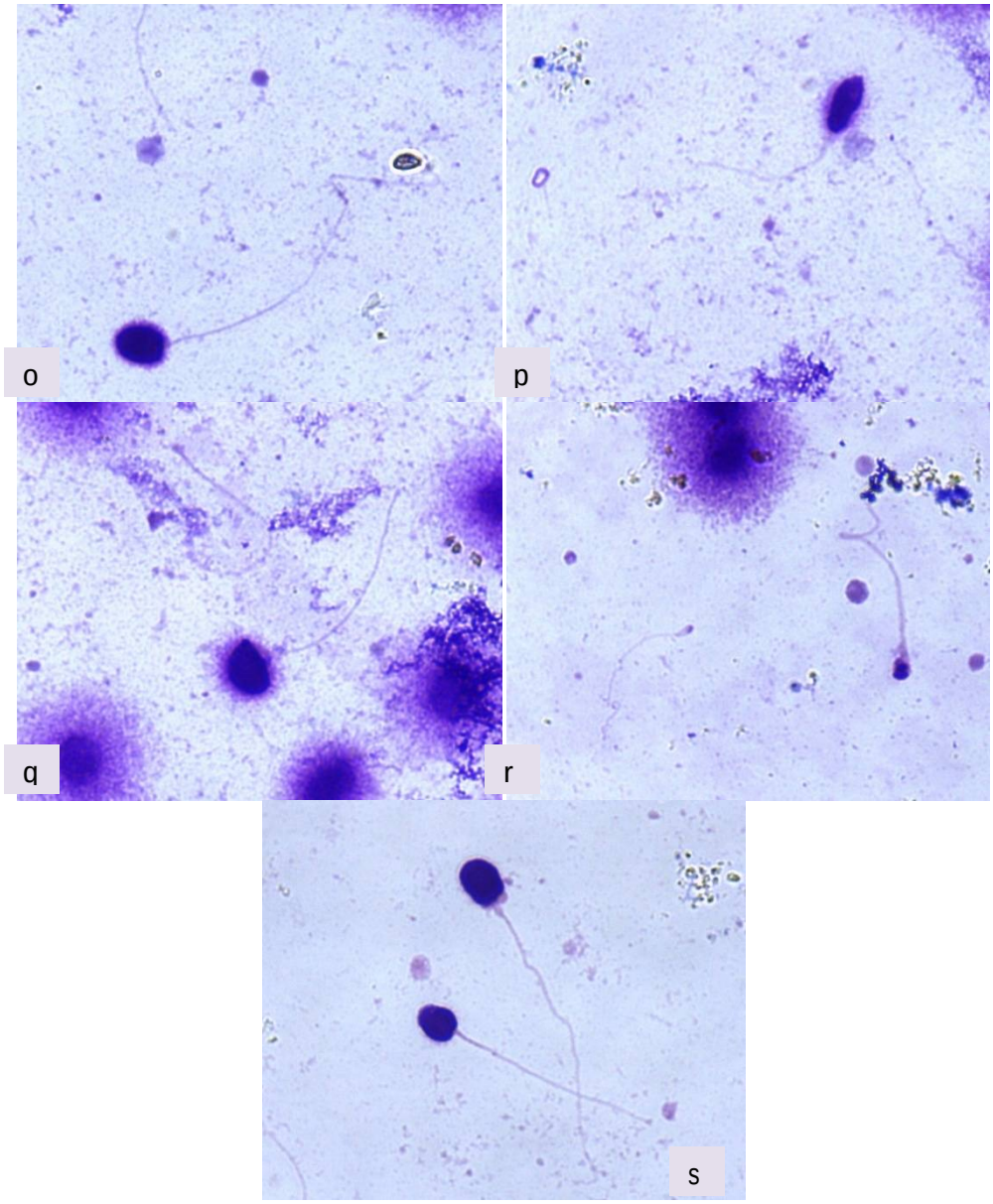

Figure (1)a-Wright staining for bright-field microscopy. Nucleoids with big halo of DNA .b- Wright staining for bright-field microscopy. Nucleoids with medium-sized halo of DNA.c- Wright staining for bright-field microscopy. Nucleoids with small-sized halo of DNA with normal head.d- Wright staining for bright-field microscopy. Abnormal sperm with small-sized halo of DNA.e- Wright staining for bright-field microscopy. Bicephalic sperm with small-sized halo of DNA.f- Wright staining for bright-field microscopy. Nucleoids with small-sized halo of DNAg-Wright staining for bright-field microscopy. Macrocephalic sperm with small-sized halo of DNA.h- Wright staining for bright-field microscopy. Abnormal head sperm without halo of DNA. i- Wright staining for bright-field microscopy. Bicephalic sperm with small-sized halo of DNA. j- Wright staining for bright-field microscopy. Small head sperm(pinhead) degraded DNA. k-Wright staining for bright-field microscopy.Bifllagelate degraded sperm.l- Wright staining for bright-field microscopy. Long amorphous head degraded sperm.m- Wright staining for bright-field microscopy. Round head degraded sperm with abnormal mid-piece. n- Wright staining for bright-field microscopy. Normal head sperm with small-sized halo of DNA. o- Wright staining for bright-field microscopy. Macrocephalic sperm with small-sized

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halo of DNA. p-Wright staining for bright-field microscopy. Long head sperm with small-sized halo of DNA. q-Wright staining for bright-field microscopy. Amorphus head sperm with small-sized halo of DNA. r-Wright staining for bright-field microscopy. Small head degraded sperm.s- Wright staining for bright-field microscopy. Amorphus head sperm without halo of DNA.

V. DISCUSSION

The most frequent cause of sperm DNA damage is the oxidative stress to which sperm exposed during its transport through the male reproductive tract ,hence sperm DNA fragmentation occur normally in male(Aitken and De Luliis ,2010 ; Sakkas and Alvarez, 2010) . The result revealed the significant difference between the control and the other two groups but no difference between the office workers and the direct exposed workers. Barrat *et al*.,(2010) found that environmental toxicants can potentially trigger sperm DNA fragmentation. The high DFI levels in both office workers and direct exposed workers ascribed to the increased levels of pollutants especially PAHs in addition to the PAHs that they exposed in their daily life including food ,water ,ambient air and smoking. Some of the PAHs are attached to the small particles of dust and therefore they reached the office workers moreover some of the office workers made regular visits to the work place. The present study showed a negative correlation between sperm DNA fragmentation and sperm motility in which the motility levels decreased as the DFI levels increased . Huang *et al*.,(2005) ; Sheikh *et al*.,(2008) ; Rafighdoost *et al*., (2012); Hassan and Mustafa , (2014);Kumar *et al*.,(2014) and Sivanarayana *et al*.,(2014) found out the association between DFI levels , motility and morphology levels except Boushaba and Belaaloui ,(2015) who also found that sperm DNA fragmentation affect the sperm motility but did not affect their morphology.

VI. CONCLUSION

Exposure to petroleum products increased different genetic abnormalities including DNA fragmentation in petroleum workers which in turn the sperm motility ,It was also found that persons with TT polymorphism is more sensitive .

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