The Effect of Chemical Weapon Exposureon Semen Fluid Analysis and the Determination of SOD and MDA Levels in Peshmerga against ISIS War

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Abstract

The identification of chemical weapons as “weapons of mass destruction” highlights their possible damaging consequences on many civilians’ health.The effect of chemical weapon agents has been documented to cause reproductive toxicity and have adverse effects on semen, leading to infertility in those exposed to those chemical agents. This study analysesthe semen fluid and follows up the exposed Peshmerga forces to the chemical weapon during the ISIS war.A prospective cohort study in which 58 exposed Peshmerga in three chemical attacks, compared with the same number of the non-exposed Peshmerga. Semen fluid analysis was performed for all, following up after one year.The serum level of both MDA and SOD was measured. An individual’s BMI was calculated.The participants’ mean agein this study is (32.5) years in the case group while (35.5) years in the control group, with a standard deviation of5 years in both groups. All participants are male, and no gender effect be seen. All chronicillnesses have been excluded from the study. The mean body mass index is 22.4, with a standard deviation of about 3.1 kg/m. Hypospermia developed in 18% of the exposed group(20% of the total participants), oligospermia (20% overall, only 3% of these in the non-exposed), and decreased motility in 19% of all participants, but 16 of this 19% where the exposed group with significant relative risk results, while morphology not changed inboth groups, also the findings showed that significant elevation in MDA level and SOD activity. Chemical weapons cansignificantly affect semen fluid analysis and interpretation through quantitativedisturbances in the volume and account of sperm, while the percentage of abnormal spermcounts has been increased considerably in peoples exposed to chemical weapons. However, the morphology of semen fluid sperms has not changed to the same degree, and its functioncannot be estimated. Even when functioning, the genetic errors on future generationsmay need further testing.

Keywords: *Exposure, Peshmerga, semen fluid analysis, MDA, and SOD.*

Introduction

Infertility has beenidentified as the failure to function to attain pregnancy one year after regular, unprotected intercourse.[1,2]In recent years, reproductive medicine and specifically male infertility have developed unexpectedly in the domain of appropriate evaluation, etiological factors such as anatomy, genetic, environmental aspects.[3]Semen analysis is very important for evaluating male infertility. Samples can be collected by masturbation after a period of abstinence of two to three days. Semen volumes and sperm concentrations might be considerably different among fertile men. Several samples might be required before it is possible to conclude that the results are abnormal.[4]Semen fluid volume greater than two ml is generally accepted as a reference value for any semen analysis, thesperm concentration ismore than 20 million/ml, total count

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more than 40 million/ejaculate, motility of morethan 50% motile, with 25% rapid progressively motile, and morphology to be more than15% normal, vitality (live) more than 75% of the collected sample, and the leukocytes mustbe less than one cell in each million/ml. A normal sperm concentration is greater than 20 million per milliliter as a result; however, men with low sperm counts may be fertile.[5]The accessory sex glands' ability to dilute the concentrated epididymal spermatozoa during ejaculation with fluids. Sperm concentration is affected by other reproductive organs' functioning, so it is not a quick indicator of testicular sperm product. Nevertheless, multiplying sperm concentration by semen volume yields the total number of sperm ejaculated.[6]In the absence of ejaculation, spermatozoa accumulate young and older adults' sperm concentrations in semen fluid, for example, maybe similar. Still, theirtotal sperm counts may vary; as people get older, both the volume of seminal fluid and total sperm production decrease, as a minimum, in some populations.[7] The time since the last sexualactivity. The epididymis is then flushed out in the urine after running into the urethra. The increased length of abstinence and penultimate abstinence period does not affect sperm vitality or chromatin.[8-10]Semen is formed during ejaculation from a concentrated suspension of spermatozoa held in the paired epididymis, fluid secretions from the accessory sex organs mix with and dilute, containing two main quantifiable attributes. The total number of spermatozoa is the first attribute, and it underlines sperm production by the testes and the post-testicular duct systems potential. The total fluid volume, which reflects secrecy activity of the glands, is the second attributed by all the accessory glands; the sperm (its vigor, motility, and morphology) is therefore of particular importance, and the composition of seminal fluid for sperm usages purposes.[6]This social problem became very easy to be solved in medicine with the help of newtechnology even though these slightly new techniques assistedvarious infertile men to be afather, the long-standing dream that they had, several other males who were fertile and lost their probablefertility as a result ofnumerouscausesfor example exposure to chemical, physical, biological andadditionalecologicalrisk agents.[3]Some men with low sperm counts are still fertile. Different typesof sperm function tests can be performed in specialized laboratories. However,they do not add much to the treatment options.[4]Etiology: toxic materials harmthe testes as well, such as insecticides,fungicides, heavy metals,cottonseed oil, and chemicalagents used during wars, which may createnegatively affected the germ cells. Leydig cells are also less susceptible than Sertoli or germ cells to the majority of chemotherapeutic medication. However, serum testosterone stages are usually used in exposed men despite their infertility.[5] However, exposure to these agents is not the only aspect that affects fertility. Suppose it is at work and to chemical agents. In that case, it will cause continuous exposure more likely; even people who live in contaminated zones could have infertility because the protection of humankind is a serious environmental crisis that can disturb this process (fertility). Such a problem cannot be terminated unless international cooperation and interference regarding this problem will be planned.[3] Chemical Conflict Agents, used in wars between Iraq and Iran (S/16433, United Nations, 1986). For example,Sulfur Mustard is a lipophilic alkylating chemical agent used as a primary chemical warfare weapon. Most of the patients have been exposed to the chemical warfare agents, whichexistinga single contactwithSulfur - mustard gas. Nonetheless,both concentration and duration of the breath of mustard diverse. As long as we were investigating the effects of chemical warfare agents and organizationover the histories of people who had been exposed, we comprisedpeople with a single exposure.[15]Terrorists could find agents problematic to employ as weapons of mass destruction for severalsimilar reasons that apply to choking agents. The effects can enter the body in various ways like breath or interaction with the eyes or the skin. Someagents can infiltratebyregular clothing materials, triggeringblisters even in parts with a cloth covering. Whereasagents that cause blisters can respondrapidly throughcontact with the skin, their symptoms could be overdue. In the agent mustard, impairmenthappenswithin 1 to 2minutes during the exposure time, choking agents are mostly gases, have noticeablesmells, and the color maysurround the air. Investigation detecting chemical agents after chemical weapon exposure is thepart of the concern for soldierlydevelopers, although some of these agents need detection proficiencies at first responders qualified to handle risky materials, whereas, some ofthe armed units have suitableequipment for chemical weapon detection, civilian people who are the first responders, they use a diversity of profitable equipment to spot and recognize an inclusivevariety of chemicals, mostly in a dangerous material background.[12,13]

Material and Methods

Patient and sampling

A descriptivecohort study is performed. In this study, we examined 63 exposed Peshmerga, 58 of them included and taken as a sample group. In contrast, 22 participants were taken from the samemilitary unit as the control group (who are not exposed). During the study, initialabnormal results from both groups are excluded (exclusion criteria). The data collecteddifficultly, and it is not far from the examiner's exposure to the remnant of chemical weaponproducts carried by the participants. However, data collection and analysis were performed inthe forensic medical institute in Arbil city from 2016 till May 2018. All victims were transferred to the local military health care unit and then transferred to the emergency hospital at the Erbil west emergency hospital. They have both signs and symptoms ofdyspnea, lacrimation, cough, skinsores, pruritus, anderythema. Samples were transferred to the forensic medicine institute, then to a specific international laboratory and exposed confirmed through examples taken from clothes, blood, urine, hair, saliva, and semen fluid sample-exposuredocumented by international labs and through the help of some humanitarianorganization. Inclusion criteria detailed chemical exposure. Asingle exposure to a different chemical agent is defined that can cause acute signs and symptoms exclusion(criteria 1)none exposed and non-documented exposure (criteria 2) history of primaryinfertility. All patientshad the same clinical severity; that is why no categorization was performed for dividingexposed military units. However, the sample was taken as the case group, and an equal number of militaryunits taken as the control group concerning age and location. Both groups areexaminedperiodically by re-taking history and re-examination with repeating semen fluid analysis (with three-day abstinence from sex beforeexamination) semen fluid analysisperformed in labs of the forensic medical Institute of Arbil city and patient-guided by expertnurses with analysis of the sample according to international guideline and standardlaboratory instruments and equipment. After the case group was exposed to the chemical warfare agent, the study group and the control group were followed up on during the study. The membersofthe control group had never been exposed to somewhat of chemicalwar agents. Both groups were nominated from a similarmilitary service location andwere told similar conditions apart from the chemical agent.

Procedure

After 30 minutes as the time of liquefaction, the sample (ejaculated semen) collected in a container-specific sterile tube, immediately taken to the lab, with labeled data, date, and time besides the name of the patient recorded on both container and visitors of the lab, for analysis, quantitative and qualitative study for semen fluid performed by an expert analyzer. Then all results were recorded and saved on the lab system for further evaluation. The method of Estimating the activity of the Superoxide Dismutase (SOD) enzyme depends on the SOD's ability to prevent epinephrine from being autooxidized into adrenochrome, the reaction occurs at 30 ºC, pH= 10.2, and SOD measured at wavelength 480nm, absorbance was measured using Spectrophotometer (G10S UV-VIS, Thermo scientific, USA).[14]Thiobarbituric acid (TBA) reacts with Malondialdehyde (MDA), the lipid peroxidation process's end product, at 100 ºC and acidic medium produce a pinkish complex product. The product is measured at 532nm. Absorbance was measured using Spectrophotometer (G10S UV-VIS, Thermo scientific, USA).[15]

Statistical Analysis

New WHO guidelines have been regarded in all aspects of the infertility questionnaire for data collection from the case and control group during the study period. All data were entered into a computer by using Excel grogram. All statistics analyzed using SPSS (Version 21) and a significant P-value of less than ($P \leq 0.05$)with 95 % confidence intervals.

Results

The average age in this study of the participants is 32.5 in the case group while 35.5 in the control group, with a standard deviation of 5 years in both groups. All participants are male, and no gender effect be seen. All chronic illnesses have been excluded from the study. The mean body mass index is 22.4, with a standard deviation of about 3.1 kg/m;nearly all exposed peoples are smokers, and all are married. Table 1 shows the effect of chemical exposure on semen fluid volume (semen volume of fewer than 2 ml is regarded as hypospermia), the incidence of hypospermia compared in both groups compared (relative risk). Hypospermia is increased in the exposed group by about 3.2 times than in the non-exposed group. The lower limit range of hypospermia recorded is 0.5 in three participants in the exposed group, with an average semen volume of about one milliliter in the exposed group with hypospermia. In contrast, the lower limit of hypospermia is 1 milliliter in the non-exposed group.

Table 1: Association between exposure status and semen volume

ExposureStatus	Semenvolume		Total
	Normal	Hypospermia	
Non exposed	20 (90.9%)	2 (9.1%)	22 (100%)
Exposed	40 (75.5%)	13 (24.5%)	53 (100%)
Total	60 (80%)	15 (20%)	75 (100%)

Table 2 shows the effect of chemical exposure on sperm count, a prominent decrease in sperm count in the exposed group on with relative increase risk of oligospermia by about 2.6 times than in the non-exposed group. The minimum sperm count is 1 million seen in two participants in the exposed group, with an average sperm count of about 6 million and a standard deviation of about 3 million.

Table 2: Association between exposure status and sperm count

ExposureStatus	SpermCount		Total
	Normal	Oligospermia	
Nonexposed	17 (89.5%)	2(10.5%)	19 (100%)
Exposed	39 (76.5%)	12 (23.5%)	51(100%)
Total	56 (80%)	14 (20%)	70 (100%)

On further evaluation, the motility also decreased in the exposed group by 2.1 times than those not exposed (Table 3). On further analysis of the exposed group of sperm motility, the effect ranges from sluggish to extreme immobility, with nearly 70% of immobility in more than half of the exposed participants. However, the ratio is more extreme, reaching 90% of immobility in the exposed group but only reaches 65% in the non-exposed group with significantly fewer immortality cases.

Table 3: Association between exposure status and sperm motility status

ExposureStatus	MotilityStatus		Total
	Normal	Immotile	
Non expose	15(88.2%)	2(11.8%)	17(100%)
Exposed	32 (78%)	9(22%)	41(100%)
Total	47(81%)	11(19%)	58(100%)

On the other hand, no effect of chemical exposure is seen according to this study on sperm morphology. The relative risk between the incidence of exposure to the non-exposed group is about equal (relative risk =1) (Table 4). The accepted lower limit of 5% normal morphology apparent in nearly all participants in both groups. Even those with abnormal morphology are a total of three participants, and one of them in the non-exposed group and has an abnormal morphology of about 4.5 %. The exposed group, on the other hand, displayed normal morphology in only two participants.

Table 4: Association between exposure status and sperm morphology

ExposureStatus	Morphology		Total
	Normal	Abnormal	
Non exposed	16(94.1%)	1(5.9%)	17(100%)
Exposed	46(95.8%)	2(4.2%)	48(100%)
Total	62 (95.4%)	3 (4.6%)	65(100%)

In contrast,Table 5 shows the results of MDA and SOD levels for patients and controls. It shows that patients' MDA levels have increased significantly (0.03558 ± 0.02012 μmol/l) compared to controls (0.006 ± 0.00285 μmol/l), at a probability ($P \leq 0.05$), respectively. It also shows showed a significant increase in SOD values of patients (0.601 ± 0.052 μmol/min/ml) compared to controls (0.924 ± 0.066 μmol/min/ml) respectively.

Table 5: MDA and SOD levels

Variants	Mean ± SD		T-test	P ≤ 0.05
	Patients	Controls		
MDA µmol/L	0.03558 ± 0.02012	0.006 ± 0.003	7.29	0.0001
SOD µmol/min/ml	0.924 ± 0.066	0.601 ± 0.052	8.84	0.0001

Discussion

Ghanei and Allameh,[14]from Iran studychemical warfare agents' effectonfertility in the case-control pattern through a questionnaire-based method to detect infertility in the exposed group versus the non-exposed group. This is in comparison to our study. We concentrate on the result of semen fluid analysis only; the comparison of each survey is to single exposure to chemical weapons with follow up in one year. The chemical materialsused in the war on Iraq-Iran were archived in the United Nations S/16433, 1986. While our article is not documented by the United Nations but supported by several humanitarian organizations, Ghanei and Allameh,[14] 2500 male studyhas participated, but in our researchare 58 males, which is a relatively smaller sample. In both mentioned studies, the presence of a prior history of infertility is an absolute criterion for excluding the exposed person or participant in the control group. The research group's average age was 28.5 +/- 4 in the Ghanei, andAllameh,[14]study versus 32.5+/- 3 in our study, when compared to the control group, the mean age of participants was 35.4+/- 5,while 38.5+/- 3 in the current study, which indicates no so much difference in the age groups in both studies, making an age effect of being not significant. At the end of the study of Ghanei andAllamehconcluded no impact of a chemical weapon on male fertility while the results on semen fluid analysis were not declared.[14] Still, in our research, it is finely analyzed the effects on semen fluid analysis,making a question for future study and the possibility of gene error detection as a plan for a prospective study.On the other hand, and in a study performed in Iran in 2005, there was no infertility registryavailableamongst the soldiers whohad been exposed to chemical war agentsthroughout the first gulf war. Male infertility was not proven, and there was no noticeable significant difference between the exposed and non-exposed soldiers.[15]In these two contrary studies, we can cross to an idea in which the functional assessment may give the exact explanation for semen fluid. However, none of these studies, including the current research, didperform this step in his article during his phases of medical works. Many chemicals in use in industry and are over the earth and within the water of use, seems to be reaching the semen fluid of the infertile men, reports bya Queen's university research scientist,[16] which may make the isolated effect of these chemical weapons of concomitant harm in both control and patient's groups, that is to say, the environmental impact of these chemicals to be of note. In patients with normal semen fluid parameters besides blood count and 24-hour urine analysis of most sensitive biologic index to think about psychiatric complain in the exposed group,[17]and may which may give an excuse for further functional testing in current and future study, In this study also, a significant increase in MDA levels was detected in the patients; this elevation in MDA levels as a result of an overabundance of reactive oxygen species (ROS) contributing to an excess of oxidative damage in these patients. In turn, these oxygen types can oxidize various other vital biomolecules, including the membrane of lipids. This rise in MDA levels corresponds to a study that found an increase in MDA levels in patients suffering from reproductive problems.[18]An elevation in SOD activity stages may be due to excessive ROS generated through oxidative stress (OS). Also, the increase in SOD activity levels could be due to the rise in OS patients with dismutation of superoxide results in the development of (H_2O_2). A study indicated that the levels of SOD activity were observed to be higher in the blood serum of male patients with reproductive problems.[18]

Conclusion

In the current study, we can conclude that chemical weapons can significantly affect semen fluid analysis and interpretation through quantitative disturbances in the volume and account of sperm. In contrast, the percentage of abnormal sperm count has markedly increased in people exposed to chemical weapons. However, the morphology of semen fluid did not change to the same degree, and its function cannot be estimated. Even when functioning, the genetic errors on future generations may need further testing.

Conflict of Interests: None.

Source of Funding: Self.

Ethical Clearance: The study was undertaken after gaining the Medical Research Center/ Hawler Medical University's ethics committee's approval.

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