

Impact of Low-Dose X-Ray Exposure on Human Sperm Motility and Morphology: An Experimental Study

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ABSTRACT

Background: X-ray radiation has been implicated in potential reproductive risks, particularly in its effects on sperm quality. However, the impact of varying doses of X-ray radiation on sperm motility and morphology remains poorly understood. This study aimed to evaluate the effects of low-dose X-ray exposure on human sperm in vitro, focusing on dose-dependent changes in motility and morphology. **Objectives:** The study aimed to (1) assess the impact of X-ray radiation on sperm motility and morphology, (2) determine the relationship between radiation dose and changes in sperm quality, and (3) compare the effects of different radiation doses with an unexposed control group. **Methods:** This experimental study exposed human sperm samples in vitro to X-ray radiation at doses of 15 mGy, 30 mGy, 50 mGy, 70 mGy, and 100 mGy, with an unexposed control group for comparison. Radiation was delivered using a CT scanner. Sperm motility and morphology were evaluated under a light microscope (400x magnification) one-hour post-exposure. Statistical analysis was performed using one-way ANOVA and post hoc tests. **Results:** Sperm motility and morphology showed significant differences ($p < 0.05$) between exposed groups and the control group. Doses of 50 mGy and above significantly reduced motility and altered morphology, while doses of 15–40 mGy caused no significant changes ($p > 0.05$). Across all exposed groups, reduced motility, viability, and morphological abnormalities were observed, with the most pronounced effects at 50 mGy or higher. **Conclusion:** X-ray radiation at doses of 50 mGy and above significantly impairs sperm motility, viability, and morphology, suggesting potential reproductive risks. Lower doses (15–40 mGy) appear to have minimal impact. These findings emphasize the importance of limiting X-ray exposure to mitigate fertility risks and highlight the need for further research on its long-term implications.

Keywords: X-Ray, Sperm Motility, Morphology

INTRODUCTION

The energy that a radiation source emits as light (photons), electromagnetic waves, or particles is known as radiation. X-rays are a type of ionizing radiation that falls within the electromagnetic wave category of short wavelengths because of their short wavelength. X-rays are a type of ionizing radiation that is used in a constantly growing number of diagnostic procedures. X-ray diagnostic imaging has been employed in the medical field for more than a century [1]. Radiation has advantages for human health, but there is also a risk. The impact of radiation on fertility is oligospermia, or a reduction in the number of sperm cells, which is already possible after radiation exposure of 0.15 mGy. This impedes the process of generating the resultant sperm cell through a deterministic effect [2]. Infertility affects 1 in 6 couples globally. Nearly 50% of infertility cases are caused by males. Although low sperm counts, abnormal sperm morphology, and impaired sperm motility are recognized causes, the underlying reasons remain unknown in 25% of instances [3]. Human infertility relies on the meiotic process, which leads to the development of germ cells. This procedure is extremely radiation-sensitive [4]. The relationship between radiation exposure and infertility is thought to originate from DNA damage induced by free radicals originating from radiation ionization [5]. DNA damage might cause some mutations or cell death (apoptosis) [6]. Although X-rays are among the most common diagnostic instruments used on patients, few studies have investigated the impacts of X-ray exposure on medical workers [7]. One condition that is commonly observed in medical workers who are exposed to radiation regularly is infertility. For the diagnosis of many illnesses, radiodiagnosis tools such as C-ARM and CT-SCAN are used [8]. X-rays are still the mainstay. Small amounts of X-ray radiation can

accumulate frequently, resulting in other abnormal epigenetic patterns and DNA damage [9, 10]. To reduce the degree of radiation exposure by medical workers, further studies are needed to determine the best X-ray dose, time, and distance [4].

Objectives

To explore how X-rays affect the motility and morphology of human sperm.

PATIENTS AND METHODS

Study Design and Participants

The laboratory design of the It is an experimental study in vitro included a control group and groups exposed to radiation that only participated in the post-test. The criteria for the people from whom samples were taken were as follows: aged 20- 50 years, aged 60- 130 kilograms, and healthy without any physical disability. Samples were obtained from the unit of infertility of Al-Batol Hospital in the Diyala Government, Iraq. There are four categories, and each sample is randomly assigned to one of them. With a minimum need of 6 samples per group, a total of 30 samples of human sperm were collected for this investigation, with 6 samples per group being used. The samples were randomly assigned to one of four groups: P1, which received X-ray radiation doses ranging from 1 to 25 mGy; P2, which received an X-ray radiation dose ranging from 26 to 50 mGy; P3, which received an X-ray radiation dose ranging from 51 to 75 mGy; and P4, which received an X-ray radiation dose ranging from 76 to 100 mGy. A negative control group that was not exposed served as the study's

control group. For human sperm cell morphology and motility analysis, sperm were collected from donors by masturbation without the use of any adjunct materials. The X-ray generator is used to produce X-ray radiation at the Al Batol hospital in the Diyala Government. The radiation exposure is carried out via CT-SCAN with the following specifications from the SIEMENS Model: Somatom Definition AS 128, X-ray tube voltage (70- 140) kV, gantry aperture (780 mm), number of slices (128), maximum patient weight (307) kg, full 360° scan time (0.28 s), and generator power (100) kW. Radiation exposure doses are determined by combining the probe's distance from the sample with the radiation apparatus's kVA/mAS output. To ascertain the best dosage equation for radiation exposure within the group, the dosimetry findings were analyzed. Sperm analysis is carried out via direct preparation in conjunction with motility testing. A 400x magnification light microscope was used to conduct the observations. The evaluation is performed quantitatively via the WHO calculation technique as the percentage of spermatozoa motility. This study uses the following classification: progressive motility (PR), in which spermatozoa in a straight line actively move; nonprogressive motility (NP), in which all forms of motility apart from progressive movement are included; slow progressive motility (SP), in which the visible movement of flagella is also included; and immotility (IM), in which spermatozoa do not move at all. Lab manuals

published by the World Health Organization in 2010 state that morphological evaluations are conducted on live spermatozoa. The quantity of viable, living, and motile spermatozoa is expressed as a percentage.

Statistical Analysis

A normality and variance test were used to analyze the sperm motility and morphological data. One-way ANOVA is the hypothesis test utilized if the data variances and distributions are equal and normal. If $p < 0.05$, the data were considered normal.

The data homogenization test is run to identify the post hoc test that will be utilized. Statistical Product for Windows software (SPSS 26) was used to analyze all computerized data processing processes.

Results

Sperm Motility

To evaluate the randomization of research subjects, a homogeneity test was performed based on body weight in this study. After randomization, body weights were normally distributed, With $p > 0.05$. Features in a group using a one-way analysis of variance (ANOVA) test are shown in this table (Table 1).

Table 1: Sample Feature

Groups	N	Mean $\pm$ SD	Normality	P - Value
Control 1	6	25.00 $\pm$ 9.441	0.937	0.368
P1	6	8.46 $\pm$ 7.723	0.888	
P2	6	20.42 $\pm$ 8.712	0.963	
P3	6	13.75 $\pm$ 5.367	0.711	
P4	6	57.38 $\pm$ 15.994	0.951	

P1: sperm exposed to (1–25) mGy X-ray radiation. P2: Sperm exposed to (25–50) mGy X-ray radiation. P3: Sperm exposed to (50–75) mGy X-ray radiation. P4: sperm exposed to (75–100) mGy X-ray radiation.

The percentage of sperm motility in each group was found to be normally distributed, with $p > 0.05$ in this research, and the Shapiro-Wilk test was used to evaluate the validity of the data using one-way analysis of variance, as in (Table 2). In addition, to determine the average difference between the groups, one-way ANOVA was used. A significant difference in means between the groups was shown by the one-way ANOVA test results (Table 3). with a p-value less than 0.05. Levene's test was used to analyze the means to compare the groups' average data for any discrepancies in variability. The findings revealed that there were no differences in variance between groups with $p > 0.05$. To examine the mean differences between the research groups, the

Bonferroni (post hoc) test was used. Compared with the control group, the spermatozoa radiation exposure group presented significantly different sperm motility ($p < 0.05$), as indicated by the Bonferroni statistical analysis findings. Additionally, groups subjected to X-ray radiation at doses ranging from 25–50 mGy, 50–75 mGy, and 75–100 mGy presented significantly different patterns of sperm motility. Figure (1) shows the variation in sperm motility in humans after exposure to X-rays compared with the control group not exposed to radiation. The Bonferroni (post hoc) test as it is pairwise comparison testing is a statistical method used to determine differences between pairs of groups after performing a one-way analysis of variance (ANOVA) test.

Table 2: Comparison of the Motility Percentage with Different Radiation Doses.

Groups	N	Mean $\pm$ SD	Normality	P value
Control 1	6	65.00 $\pm$ 4.472	0.167	0.000343*
P1	6	53.83 $\pm$ 12.254	0.675	
P2	6	35.00 $\pm$ 17.607	0.248	
P3	6	31.67 $\pm$ 8.165	0.505	
P4	6	50.83 $\pm$ 14.972	0.378	

* Indicates that the mean difference is significant at the 0.05 level. P1: sperm exposed to (1–25) mGy X-ray radiation. P2: Sperm exposed to (25–50) mGy X-ray radiation. P3: Sperm exposed to (50–75) mGy X-ray radiation. P4: sperm exposed to (75–100) mGy X-ray radiation.

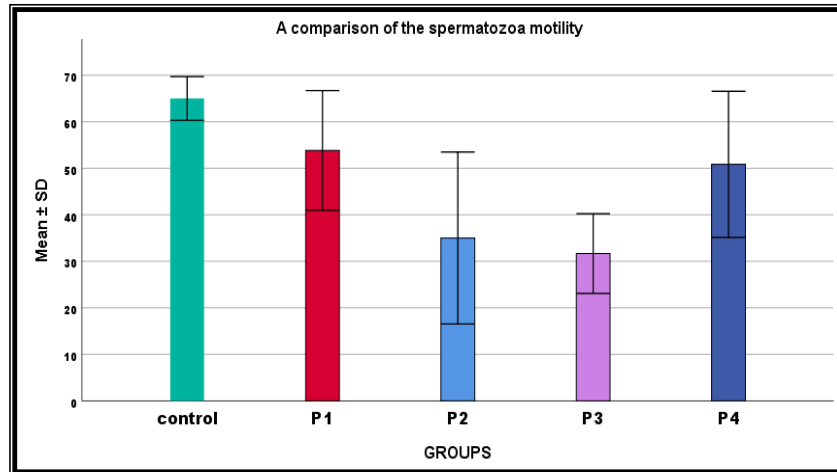
Table 3: (a) Variation in the Percentage of Sperm Motility with Different Radiation Doses.

Comparisons Between Groups of Sperm Motility	Confidence Interval 95%			P-value
	Difference Mean	Lower Bound	Upper Bound	
Control vs P1	11.167	-3.60	25.93	0.132
Control vs P2	30.000	15.23	44.77	0.000*
Control vs P3	33.333	18.57	48.10	0.000*

Table 3: (b) Variation in the Percentage of Sperm Motility with Different Radiation Doses.

Comparisons Between Groups of Sperm Motility	Confidence Interval 95%			P-value
	Difference Mean	Lower Bound	Upper Bound	
Control vs P4	14.167	-.60	28.93	0.059
P1 vs P2	18.833	4.07	33.60	0.015*
P1 vs P3	22.167	7.40	36.93	0.015*
P1 vs P4	3.000	-11.77	17.77	0.679
P3 vs P4	19.167	1.07	30.60	0.013*
P2 vs P4	15.833	4.40	33.93	0.037*

* Indicates that the mean difference is significant at the 0.05 level. P1: sperm exposed to (1–25) mGy X-ray radiation. P2: Sperm exposed to (25–50) mGy X-ray radiation. P3: Sperm exposed to (50–75) mGy X-ray radiation. P4: sperm exposed to (75–100) mGy X-ray radiation.

**Figure 1:** Variation in Human Spermatozoa Motility After Exposure to X-Rays

Overall, the data revealed that there were few statistically significant differences between the groups being compared by using a one-way analysis of variance (ANOVA).

Indicating that there were significant effects on sperm motility between the different experimental groups exposed to X-rays and the control group shown in Figure 1.

Sperm Morphology

After conducting a statistical test to establish the normality of the morphology data collected from microscopic observations, the Shapiro–Wilk normality test revealed that the data were normally distributed. with $p > 0.05$. These data are displayed by using a one-way analysis of variance (ANOVA) in Table 4.

Table 4: Variation in Normal Sperm Morphology Upon Radiation Exposure.

Groups	N	Mean ± SD	Normality	P Value
Control 1	6	75.00 ± 3.162	0.827	0.101
P1	6	63.33 ± 12.517	0.847	
P2	6	35.00 ± 18.439	0.963	
P3	6	37.83 ± 11.232	0.865	
P4	6	46.67 ± 15.384	0.831	

* Indicates that the mean difference is significant at the 0.05 level. P1: sperm exposed to (1–25) mGy X-ray radiation. P2: Sperm exposed to (25–50) mGy X-ray radiation. P3: Sperm exposed to (50–75) mGy X-ray radiation. P4: sperm exposed to (75–100) mGy X-ray radiation

Following the results of the normality test, which revealed that the morphological distribution was normal, a parametric one-way ANOVA test was run. A significant difference ($p < 0.05$) in the means between each group was detected. The data variability was examined via the Levene test, and homogenous data were identified when $p > 0.05$. Therefore, the LSD test was performed after the test. A variation with statistical significance ($p < 0.05$) was detected in the mean spermatozoa morphology between the control and exposed groups. The groups that received radiation from 25–50 mGy X-rays differed from the groups that received 50–75 mGy and 75–100 mGy X-rays, with $p < 0.05$. After further analysis, $p > 0.05$ revealed no

discernible differences between the groups that received X-ray exposure to (75–100) mGy and those that received (50–75) mGy the data shown by using the Bonferroni (post hoc) test as it is pairwise comparison testing is a statistical method used to determine differences between pairs of groups after performing a one-way analysis of variance (ANOVA) test shown in (Table 5). There were statistically significant differences between the control group and several experimental groups, as well as between some of the experimental groups themselves, as shown in Table No. (5) and Figure No. (2). These differences indicate clear effects of exposure to different doses of X-rays on sperm morphology.

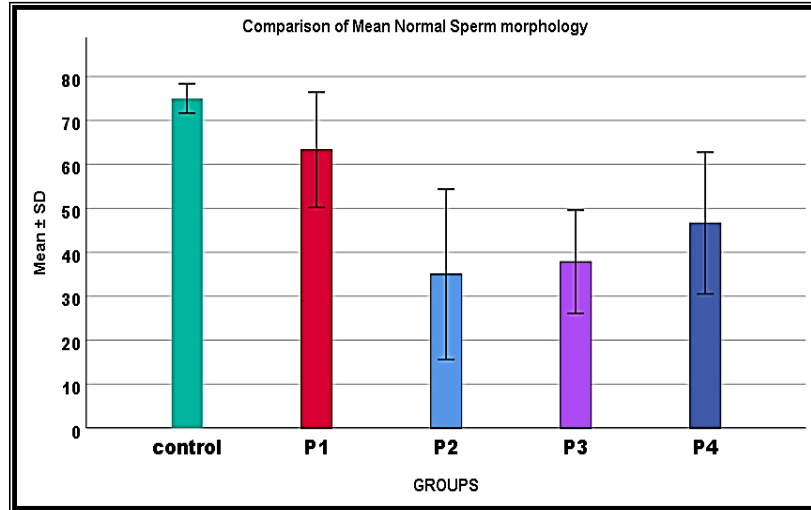
Table 5: (a) The Proportion of Sperm Exhibiting Normal Morphology Differed Among the Groups Exposed to Varying Levels of Radiation.

Comparisons between Groups of Sperm Morphology	Confidence Interval 95%			P Value
	Difference Mean	Lower Bound	Upper Bound	
Control vs P1	11.6667	-4.0138	27.3471	0.1380
Control vs P2	40.0000	24.3195	55.6805	0.0000*
Control vs P3	37.1667	21.4862	52.8471	0.0001*

Table 5: (b) The Proportion of Sperm Exhibiting Normal Morphology Differed Among the Groups Exposed to Varying Levels of Radiation.

Comparisons between Groups of Sperm Morphology	Confidence Interval 95%			P Value
	Difference Mean	Lower Bound	Upper Bound	
Control vs P4	28.3333	12.6529	44.0138	0.0010*
P1 vs P2	28.3333	12.6529	44.0138	0.0010*
P1 vs P3	25.5000	9.8195	41.1805	0.0026*
P1 vs P4	16.6667	0.9862	32.3471	0.0381*
P3 vs P4	8.8333	-6.8471	24.5138	0.2569
P2 vs P4	11.6667	-4.0138	27.3471	0.1380

* Indicates that the mean difference is significant at the 0.05 level. P1: sperm exposed to (1–25) mGy X-ray radiation. P2: Sperm exposed to (25–50) mGy X-ray radiation. P3: Sperm exposed to (50–75) mGy X-ray radiation. P4: sperm exposed to (75–100) mGy X-ray radiation.

**Figure 2:** Variation in the Mean Sperm Morphology After Exposure to X-rays.

Statistical method used to determine differences between pairs of groups after performing a one-way analysis of variance (ANOVA) test in Figure 2.

Discussion

The present study revealed a difference before and after X-ray exposure in the sperm of the normozoospermic group [11, 12]. The motility of sperm decreases significantly after exposure to a dose above 50 mGy and can be considered significant. It is important to know some characteristics when studying sperm, the most important of which is "The singular form of the medical term "spermatozoa" is spermatozoa. A process known as "spermatogenesis" produces spermatozoa, male gametes or reproductive cells; in the testis, the primary male sexual organ or gonad. Each single sperm is a single haploid cell. Sperm cells have a finite life span and are not able to divide. The zygote grows into a new organism after it fuses with egg cells [13]. During ejaculation, male gametes called sperm are produced together with nutritional fluids. The male reproductive system releases seminal fluids and sperm during ejaculation. The typical human ejaculate contains sperm cells. The male testicles generate thousands of sperm each day. They are fed and grow in the testis's ejaculation. The quantity of sperm in one milliliter of ejaculation is known as the sperm count. One hundred million sperm typically make up 1 milliliter of sperm. Three milliliters of sperm are typically discharged during ejaculation. Consequently, the ejaculation will include 300 million sperm in 3 mL [14]. A single sperm has approximately 3.3 billion base pairs of DNA, and every base pair consists of two bits of information, making each sperm 787 Mb in size. Approximately 280 million sperm are produced during ejaculation, meaning that a single load would hold nearly 220,000 terabytes of data

[15]. This study focused on two important parts of human sperm exposed to X-rays. Sperm motility was classified as progressive, nonprogressive, slow-progressive, or immotile motility, and morphology was classified as normal or abnormal. Initially, thirty human sperm samples were taken from healthy individuals; then, a sample analysis (SFA) was performed for all the samples before irradiation, and the information about each donor was confirmed. The next step involved exposing the samples to X-rays at a dose of 25 mGy, after which they were returned to the storage container at body temperature or slightly higher [16, 17]. After one hour, the samples were examined again. There was no significant difference after irradiation, and sperm movement was normal ($P > 0.05$). Compared with that before irradiation, no noticeable change in shape was observed under an optical microscope. The experiment was repeated with the remaining semen samples. An increase in the dose of X-ray exposure to 50 mGy was observed. Although a higher dose was given, no noticeable change was detected when the samples were examined after exposure to X-rays. Statistically significant differences were recorded in grade (D), but there were no statistically significant differences in the other grades (A, B, C). In terms of morphology, there is a significant difference between the typical and abnormal potential of sperm before and after radiation exposure. The process was repeated with the remaining samples, which were divided into two groups of 6 samples each [18].

These groups were then exposed to two different doses, 75 mGy and 100 mGy. Post radiation effects were measured for a dose of 75 mGy and 3.8 and 3.9 for a dose of 100 mGy. The results are compared. At 75 mGy, there was a noticeable effect on sperm motility, especially at Grade C, as they either had slow progressive motility or nonprogressive motility. At a dose of 100 mGy, a very significant decrease in the sperm

concentration can be observed after exposure to a dose of 100 mGy in grades A and B, which indicates a decrease in the number of rapidly and moderately progressing sperm. A decrease in concentration was observed in Grade C, but the change was not significant. The sperm morphology before and after exposure to a dose of 100 mGy was evaluated. The statistical analysis revealed a very noticeable decrease in the concentration of normal sperm. Moreover, there was a significant increase in the abnormal concentration of sperm after irradiation. The mechanism of the effect of X-rays on the movement and shape of human sperm can be explained as follows: as sperm cells absorb the energy of ionizing rays falling on them, this energy is distributed throughout the cells, leading to the emergence of various activities within them. There are two types of effects on cells: direct lethal effects, which affect sensitive parts of the cell, and indirect nonlethal effects, which affect sensitive components in the cell, such as the cell wall. The bulk of the sperm is water. In addition, sperm contain hundreds of different proteins and fat, salt, calcium, fructose, and lactic acid.

Magnesium, zinc, and potassium. The presence of water makes the cell sensitive to radiation. As water molecules are exposed to ionizing radiation, they ionize and lead to the production of the free radicals $^{\circ}\text{H}$ and $^{\circ}\text{OH}$, which causes changes in the DNA and RNA of the sperm cell, which play major roles in facilitating interactions with cell components and thus disrupting their functions [19]. When the temperature ranges from 37°C to a few degrees Celsius, the sperm becomes active after ejaculation. A phenomenon called "thermal shock" occurs when the temperature increases to a point where sperm can continue to exist without being harmed [20]. When sperm cells are exposed to X-rays, the water molecules inside the cells are ionized, causing the electrical rate preserved in human sperm to quickly disappear. Sperm motility is affected by this depletion, which reduces the percentage of motile sperm, ultimately leading to reduced sperm viability [21]. In response to exposure to ionizing radiation, sperm cells can also initiate apoptotic pathways as a defense mechanism against DNA damage or physiological stress [22]. Sperm cells that have been permanently damaged or that are making an effort to overcome damage due to radiation exposure undergo apoptosis, which prevents genetic abnormalities from being transmitted to offspring [23].

Conclusion

Based on the above results, doses ranging from 1 to 50 mGy had no significant effect on sperm motility. Owing to the superior ability of cells to repair damage and withstand the effects of radiation, the damage resulting from exposure to X-rays is bypassed; in other words, the extent of damage is relatively small compared with the number of cells present. There is a direct relationship between the amount of radiation exposure and the level of resulting cell damage, which increases as the dose of radiation to which the sperm is exposed increases.

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Acknowledgments

The author expresses his profound gratitude to Prof. Dr. Aedah Zeki Alkaisy for her astute counsel and helpful critique, which prevented me from making many blunders. Additionally, I want to express my gratitude to Dr. Hussein K. Al-Dulaimi for giving his time to assist me in finding study subjects and finishing all of my assignments.

Ethical Issues

The Ethical Committee of the College of Medicine, University of Baghdad followed the Helsinki and Tokyo Guidelines for Human Subjects (Ref. No. 153 dated 1/7/2024), which approved the study protocol. In addition to receiving comprehensive information about the study, participants were asked to fill out a questionnaire that included details about their medical history and personal background. Ethical issues (including plagiarism, data falsification, and double-publishing) were fully resolved by the authors. This study is a university thesis in medical physics by the writer Haider M. Al-Khalidi at this university.

Limitations of the Study

Limited generalizability: This study used human sperm in vitro, which may not fully reflect the complexities and conditions of sperm in vivo or clinical settings.

Small Sample Size: The number of samples and participants in each exposure group may have been insufficient to detect subtle differences or to generalize findings to broader populations.

Short postexposure evaluation: The assessment of sperm motility, morphology, and viability was conducted only one hour after exposure. Longer-term evaluations could provide insights into the persistence or recovery of sperm parameters over time.

Key Point

This study investigated the effects of X-ray irradiation on the morphology and motility of sperm. The sperm motility and morphology did not significantly change at doses up to 25 mGy ($p > 0.05$). The sperm shape was unaltered at 50 mGy, but motility was dramatically reduced. As the dose increased, motility decreased noticeably. The 75 mGy dose had the greatest effect on nonprogressive or slow-moving sperm, and the 100 mGy dose had the greatest effect on the motility of high-quality sperm (A and B). X-rays can harm sperm directly by causing damage to cell components or indirectly by causing free radicals to form from ionized water within the sperm cells. In addition to causing harm to sperm, thermal shock may also trigger programmed cell death in an attempt to prevent the transmission of genetic abnormalities. Finally, whereas sperm motility is impaired at doses up to 50 mGy and has no effect on motility, larger doses result in increased exposure-related damage.

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