

ORIGINAL ARTICLE**Impact of Human Papillomavirus (HPV) on Sperm Parameters:
Role of Oxidative Stress in Semen Quality**Alaa HACHEM^{1*}, Asaad AL-SHOUK², Seemaa HACHIM³

¹*Lecturer, Department of Anatomy, College of Medicine, University of Al-Qadisiyah, Diwaniyah City, Iraq. ORCID: 0000-0002-8440-0743*

²*Assistant Professor, Department of Anatomy, College of Medicine, University of Al-Qadisiyah, Diwaniyah City, Iraq. ORCID: 0000-0003-4003-9500*

³*Lecturer, General Directorate of Education in Al-Qadisiyah Governorate, Diwaniyah City, Iraq.
ORCID: 0009-0009-6375-0638*

Correspondence: alaa.hachem@qu.edu.iq

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ABSTRACT

OBJECTIVE: To study the impact of HPV infection and oxidative stress on normal sperm parameters.

METHODOLOGY: This case-control study was conducted in the Department of Dermatology and Venerology at the Teaching Hospital of Hilla, Hilla City, Iraq, from March 2023 to October 2024. The study recruited 70 male patients diagnosed with HPV infection and 70 HPV-negative volunteers between the ages of 18 and 45. HPV infection was confirmed by real-time PCR for viral DNA. Sperm functional characteristics were assessed on a computer-based sperm analyzer (CASA). Oxidative stress in semen was evaluated by measuring levels of ROS using 2',7'-dichlorofluorescein diacetate (DCFH-DA) fluorescence assay, the 8-hydroxy-2'-deoxyguanosine (8-OHdG) by ELISA assay, and the malondialdehyde (MDA) following the thiobarbituric acid assay.

RESULTS: The percentages of sperm total motility, progressive motility, and normal morphology were significantly reduced among HPV patients ($p < 0.001$). Seminal levels of ROS, 8-OHdG, and MDA were significantly higher ($p < 0.001$) in HPV patients compared to the control group. Sperm characteristics were inversely correlated with levels of oxidative stress markers. The highest impact of the viral infection on sperm parameters and on ROS levels was identified in patients with high-risk HPV (HR-HPV) genotype (29 (41.4%)) compared to (41 (58.6%)) of HPV low-risk (LR-HPV), ($p < 0.001$).

CONCLUSION: Patients with HPV infection display elevated oxidative stress and reduced semen quality. The inverse association between oxidative stress markers in HPV patients and semen parameters indicates that oxidative stress might contribute to male reproductive dysfunction.

KEYWORDS: Human papillomavirus; Sperm parameters; Oxidative stress; High-risk HPV; reactive oxygen species; 8-hydroxy-2'-deoxyguanosine; Malondialdehyde.

INTRODUCTION

Human papillomavirus (HPV) is a very common sexually transmitted disease¹. It is caused by infection with the Papilloma virus, a DNA virus that infects skin and mucous membranes and, in severe cases, may lead to the development of epithelial tumors². Although considerable attention has been directed towards the carcinogenic properties associated with HPV high-risk strains, especially with cervical cancer, emerging studies suggest that HPV harms male reproduction³. Studies reported altered sperm functional parameters in HPV infected patients, characterized by low sperm motility, abnormal morphology, and decreased sperm count². The disease was also shown to affect the quality of seminal fluid, including viscosity, pH, and the production of anti-sperm antibodies⁴. Clinical studies linked the adverse effects of HPV infection to higher rates of miscarriage, impaired embryonic development, and reduced outcomes from assisted reproductive technologies⁵. The most pronounced impact on sperm characteristics was observed in patients infected with high-risk (HR-HPV) genotypes, specifically HPV16 and HPV18^{6, 7}, compared to low-risk (LR-HPV) genotypes, which have been associated with less impact on semen quality². Although it is becoming increasingly evident that HPV negatively impacts male fertility, the precise mechanisms affecting sperm characteristics remain poorly understood⁸. It is suggested that HPV infection imposes significant damage on the spermatogenesis process through the generation of higher oxidative stress in the seminal fluid of the infected individuals⁹. Semen from those patients showed increased levels of ROS and lipid oxidation, as well as higher levels of inflammatory cytokines^{1, 10}. Oxidative stress results from excessive formation of reactive oxygen species (ROS) in semen, which in HPV patients was associated with increased lipid peroxidation, DNA fragmentation, and protein dysfunction¹¹. Despite the large number of published studies on the impact of HPV on male fertility, multiple reports have challenged these findings^{12, 13}, necessitating further research to determine the exact role of HPV infection in male infertility. Accordingly, this study aimed to evaluate the relationship between HPV infection, oxidative stress markers, and semen quality parameters.

METHODOLOGY

The current study included 70 male patients diagnosed with HPV infection, who were randomly selected from a pool of patients attending the Department of Dermatology and Venerology at the Teaching Hospital of Hilla, Hilla City, Iraq, between March 2023 and October 2024. The control group consisted of 70 HPV-negative patients, randomly selected and recruited from the same department, who were seeking medical consultation for benign and non-infectious skin conditions such as eczema, vitiligo, seborrheic keratosis, or benign melanocytic nevi. These subjects voluntarily provided semen samples and relevant medical history. Random sampling was achieved first by identifying participants who met the inclusion and exclusion criteria, followed by generating a computer-generated random list to select individuals for both groups. Inclusion criteria for the HPV-positive group included male patients within the age range of 18 to 45 years, diagnosed with anogenital warts, and confirmation of the presence of the viral DNA following the diagnostic application of RT-PCR. The inclusion criteria of the HPV-negative group (control) included males within the age range of 18 to 45 years, with no history of HPV infection or HPV treatment, no history of infertility or infertility-related treatments, negative for HPV DNA by RT-PCR, and having fathered at least one child. Exclusion criteria for both groups included a history of acute or chronic diseases known to reduce semen quality, such as varicocele, orchitis, sexually transmitted viral or bacterial diseases, hormonal irregularities, and patients on vitamins or antioxidant supplements. In addition, those with prior administration of antiviral, antibacterial, or hormonal therapies in the past three months were also excluded.

The study received approval from the Ethics Committee at the College of Medicine, University of Al-Qadisiyah, Iraq, on the 12th of March 2023 (reference no. 77/301). All participants were fully informed, and written consent was obtained from all subjects. Collection of personal information and biological samples was carried out in accordance with the ethical principles of the Helsinki Declaration.

For HPV detection and genotyping in semen samples, genomic DNA was isolated using the instruction protocol of QIAamp® DNA Mini Kit (Qiagen, USA) according to the manufacturer's instructions. Viral DNA was detected with Norgen's HPV (High and Low Risk) End-Point PCR Kit (Norgen, Canada). The kit can detect LR-HPV and HR-HPV types in a real-time PCR (RT-PCR) test following the use of TaqMan® technology on a Qiagen Rotor-Gene Q machine (Qiagen, USA).

Semen collection and analysis were conducted according to the World Health Organization (WHO) guidelines¹⁴. Patients and control subjects were asked to abstain for 3 days sexually, then semen samples were collected by masturbation and kept at 37°C for 30 minutes to liquefy. Semen parameters, including concentration, motility, and morphology, were quantified using a computer-assisted sperm analysis (CASA) system (ASCEN Technology, China).

Measuring ROS was conducted according to **Benedetti *et al.***¹⁵. In brief, 90 µL of semen was mixed with 10 µL of 1 mM 2',7'-dichlorofluorescein diacetate assay (DCFH-DA, Sigma, Germany) in a 96-well microplate, then incubated for 30 minutes at 37°C with gentle shaking at regular intervals. Semen levels of ROS were quantified by measuring reactive fluorescence on a plate reader (Bio-Rad, USA) with excitation/emission of 485nm / 520nm, respectively.

Measuring levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG) and malondialdehyde (MDA) were achieved on isolated seminal plasma by centrifuging at 300 g for 10 minutes from whole semen. Collected seminal plasma samples were then aliquoted and stored at -80°C until used. Oxidative damage to sperm DNA was evaluated following the use of the 8-OHdG ELISA assay kit (Eagle

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Biosciences, USA). In brief, 50 μ L of prepared standards and samples of seminal plasma were pipetted into each well of the 96-well microwell plate provided with the kit in triplicate, followed by 50 μ L of antibody, and the plate was mixed well. The plate was then covered and incubated at 25°C for 1 hour. A washing step included washing wells 4 times with the provided wash buffer before adding 100 μ L of the chromogenic substrate (TMB substrate). The plate was then covered and kept in the dark for 30 minutes at 25°C. 100 μ L of the stop solution was added before measuring light absorbance on a plate reader (Bio-Rad, USA) at 450 nm. Seminal 8-OHdG (ng/ml) was quantified by measuring optical density from each sample, which was plotted against the regression formula of the standard curve.

MDA levels were assessed following the thiobarbituric acid assay, according to Hsieh *et al.*¹⁶. In brief, 100 μ L of thawed seminal plasma was pipetted into a glass tube, then diluted in 900 μ L of deionized water. Thiobarbituric acid reagent was made, which is composed of 0.67 grams of 2-thiobarbituric acid diluted in 100 ml of deionized water, followed by the addition of 0.5 grams of NaOH and 100 ml of glacial acetic acid. Half a milliliter of the reagent was added to each tube, and the tubes were incubated for 1 hour in a water bath at boiling temperature. Tubes were allowed to cool before centrifugation at 4000 g for 10 minutes. MDA level (nmol/mL) was calculated for each sample from the supernatant absorbance at 534 nm on a spectrophotometer (Biobase, China).

Statistical analyses were performed on the Statistical Package for the Social Sciences (SPSS, V. 29, IBM-USA). Power analysis was used to determine the sample size for each group, with power = 0.8, effect size = 0.5, and P = 0.05. Quantitative variables were expressed as mean \pm standard deviation (S.D.), and an independent-samples t-test was used to compare these variables. The impact of oxidative stress and HPV infection on semen characteristics was assessed using Pearson's correlation coefficient (r). Statistical significance was set at p < 0.05. GraphPad Prism statistical analysis software (v. 10) was used to generate boxplot graphs.

RESULTS

The comparative analysis of semen parameters and oxidative stress between HPV-negative and HPV-positive individuals is presented in **Table I**. Semen analysis revealed a significant reduction in total motility ($39.04\% \pm 19.52$ vs. $58.14\% \pm 23.92$, $p < 0.001$) and progressive motility ($13.12\% \pm 9.26$ vs. $29.43\% \pm 15.21$, $p < 0.001$) in the HPV-positive group compared to the HPV-negative one. The HPV-positive group also exhibited a markedly higher percentage of immotile spermatozoa ($60.96\% \pm 19.52$ vs. $41.86\% \pm 23.92$, $p < 0.001$), whereas semen volume ($p = 0.07$) and sperm concentration ($p = 0.056$) did not differ significantly between groups.

Investigating oxidative stress markers in semen of HPV-positive patients showed higher ROS levels (3826.61 ± 1702.38 RFU) compared to the control group (1540.93 ± 583.10 RFU) ($p < 0.001$). 8-OHdG and MDA levels were also elevated in HPV-positive individuals (24.29 ± 9.74 ng/ml vs. 11.75 ± 7.23 ng/ml, $p < 0.001$, and 4.16 ± 1.39 nmol/ml vs. 2.36 ± 1.45 nmol/ml, $p < 0.001$, respectively). **Table I and Figure 1**

The correlation analysis in **Table II** showed that HPV was negatively correlated with progressive sperm motility ($r = -0.55$, $p < 0.001$), total motility ($r = -0.40$, $p < 0.001$), and normal morphology ($r = -0.47$, $p < 0.001$). The correlations between HPV and semen volume ($r = -0.15$, $p = 0.07$) and sperm concentration ($r = -0.16$, $p = 0.056$) were not statistically significant. Additionally, ROS and 8-OHdG exhibited significant negative correlations ($p < 0.001$) with progressive motility ($r = -0.33$, $p < 0.001$ and $r = -0.41$, respectively), total motility ($r = -0.24$, $p = 0.005$ and $r = -0.29$, $p < 0.001$, respectively), and normal morphology ($r = -0.33$, $p < 0.001$ and $r = -0.24$, $p = 0.005$, respectively). Additionally, higher MDA levels were significantly correlated with reduced progressive motility ($r = -0.27$, $p = 0.002$) and decreased total motility ($r = -0.20$, $p = 0.019$). Current findings indicate a direct detrimental impact of oxidative markers on the quality of spermatozoa in patients with HPV infection.

Furthermore, HPV genotypic analysis revealed that HR-HPV variants had of greater impact on semen quality and oxidative stress than LR-HPV variants, as shown in Table 3. Total motility ($29.15\% \pm 17.42$ vs. $46.03\% \pm 18.00$, $p < 0.001$) and progressive motility ($7.17\% \pm 6.79$ vs. $17.33\% \pm 8.48$, $p < 0.001$) were significantly lower in the HR-HPV group. The percentage of immotile sperm was found to be higher in patients with HR-HPV ($70.85\% \pm 17.42$ vs. $53.97\% \pm 19.40$, $p < 0.001$). ROS levels were also significantly elevated in the HR-HPV group compared to the LR-HPV group (4215.22 ± 1775.46 RFU vs. 3277.21 ± 1450.77 RFU, $p = 0.018$). However, no significant differences in 8-OHdG or MDA levels were observed between the two groups.

Table I: Semen characteristics and oxidative stress markers between HPV-negative and HPV-positive groups

Parameters	HPV negative (n=70)	HPV positive (n=70)	P-value
	Mean ± S.D.	Mean ± S.D.	
Age (years)	36.09 ± 8.18	38.03 ± 8.52	0.171
Semen volume (ml)	3.08 ± 1.05	2.75 ± 1.11	0.070
Concentration (million/ml)	46.71 ± 21.4	40.0 ± 19.81	0.056
Total motility (%)	58.14 ± 23.92	39.04 ± 19.52	<0.001***
Progressive motility (%)	29.43 ± 15.21	13.12 ± 9.26	<0.001***
Non-progressive motility (%)	28.72 ± 13.06	25.91 ± 14.60	0.233
Immotile sperm (%)	41.86 ± 23.92	60.96 ± 19.52	<0.001***
Normal Morphology (%)	12.17 ± 5.58	7.03 ± 3.93	<0.001***
ROS (RFU)	1540.93 ± 583.10	3826.61 ± 1702.38	<0.001***
8-OHdG (ng/ml)	11.75 ± 7.23	24.29 ± 9.74	<0.001***
MDA (nmol/ml)	2.36 ± 1.45	4.16 ± 1.39	<0.001***

RFU: Relative fluorescence unit; ***: Significant at $P < 0.001$.

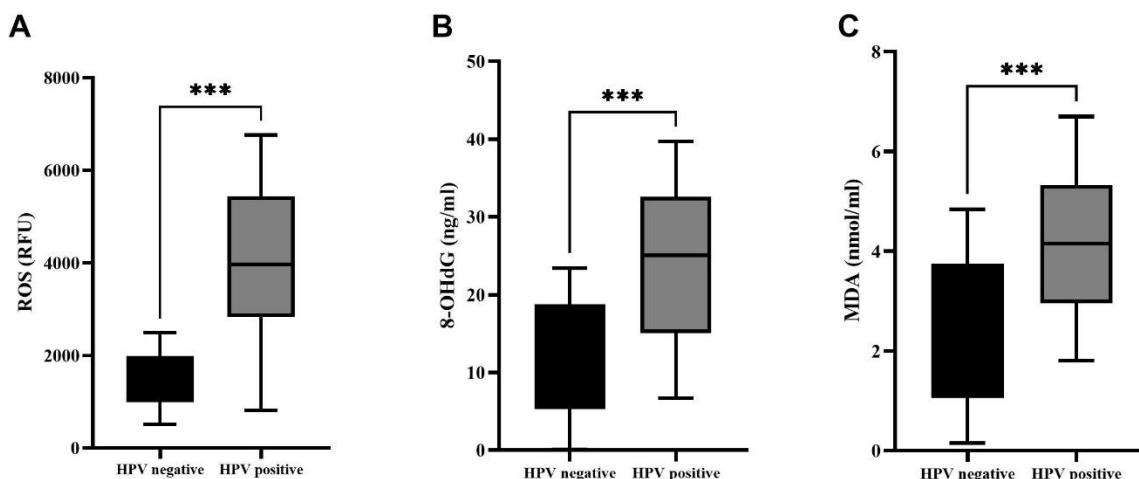
**Figure 1: Boxplots show differences in ROS, 8-OHdG, and MDA levels between HPV-negative and HPV-positive participants.**

Table II. Correlation between HPV Status, Oxidative Stress Markers, and Semen Parameters

Factors		Semen volume	Sperm concentration	Sperm total motility	Sperm progressive motility	Sperm Normal Morphology
HPV	r	-0.15	-0.16	-0.4	-0.55	-0.47
	p	0.07	0.056	<0.001***	<0.001***	<0.001***
ROS	r	-0.110	-0.03	-0.24	-0.330	-0.330
	p	0.209	0.686	0.005**	<0.001***	<0.001***
8-OHdG	r	0.110	-0.14	-0.29	-0.410	-0.240
	p	0.208	0.105	<0.001***	<0.001***	0.005**
MDA	r	-0.130	-0.19	-0.20	-0.270	-0.150
	p	0.129	0.021*	0.019*	0.002**	0.073

*Significant at $P < 0.05$; **Significant at $P < 0.01$; ***Significant at $P < 0.001$.

Table III: Comparison of Semen Parameters and Oxidative Stress Markers Between Low-Risk and High-Risk HPV Variants

Parameters	HPV low-risk variant	HPV high-risk variant	P-value
	(n=41)	(n=29)	
	Mean \pm S.D.	Mean \pm S.D.	
Age (years)	36.76 \pm 8.51	39.83 \pm 8.34	0.138
Semen volume (ml)	2.68 \pm 1.04	2.84 \pm 1.04	0.561
Concentration (million/ml)	43.87 \pm 18.16	34.52 \pm 21.04	0.051
Total motility (%)	46.03 \pm 18.0	29.15 \pm 17.42	<0.001***
Progressive motility (%)	17.33 \pm 8.48	7.17 \pm 6.79	<0.001***
Non-progressive motility (%)	28.69 \pm 13.6	21.98 \pm 15.29	0.064
Immotile sperm (%)	53.97 \pm 19.4	70.85 \pm 17.42	<0.001***
Normal Morphology (%)	7.32 \pm 4.14	6.62 \pm 3.63	0.469
ROS (RFU)	3277.21 \pm 1450.77	4215.22 \pm 1775.46	0.018*
8-OHdG (ng/ml)	23.82 \pm 9.04	24.96 \pm 10.79	0.646
MDA (nmol/ml)	4.21 \pm 1.42	4.08 \pm 1.37	0.714

*Significant at $P < 0.0$; ***: Significant at $P < 0.001$.

DISCUSSION

Findings from the current study revealed significant associations between HPV infection, reduced semen quality, and elevated oxidative stress markers in the semen. The impact of the viral infection on semen quality, such as total motility, progressive motility, and normal morphology, was significant in this study. These findings are aligned with several studies which reported several detrimental changes in sperm quality as a result of the HPV infection, including generation of oxidative stress-induced sperm impairment, mitochondrial dysfunction, dysregulation of ion channels, and structural abnormalities in the sperm flagellum¹⁷⁻²⁰. Additional research has described excessive production of anti-sperm antibodies against viral proteins in the semen of infected individuals, leading to higher viscosity, reduced pH, and impaired motility²¹. Such detrimental impacts on semen quality could significantly contribute to a higher risk of male infertility, where many clinical studies confirmed the direct effect of the viral infection on normal sperm parameters²². Unlike our results and the findings from many other studies supporting a role for HPV infection in altering normal sperm parameters, earlier studies reported conflicting findings and challenged any impact of HPV on male fertilizing ability^{12, 13}.

In this study, our patients suffered reduced total motility and lower progressive motility compared to the group of healthy volunteers. Consistent with these results, most of the previously published studies associated reduced sperm motility with HPV infection^{3, 6, 17}. Although the exact molecular impact of HPV infection on sperm motility is still not completely understood, recent research by Pellavio et al. showed that HPV infection induces inhibition of aquaporin-8, which is a water channel protein involved in the transport of water and other small molecules in sperm cells, rendering sperm more prone to oxidative damage generated by the viral infection⁸. In addition, the effect of HPV infection on sperm motility was found to be notably higher in patients diagnosed with HR-HPV genotypes, which were shown to reduce sperm motility by up to 55% compared to non-infected samples⁶. Our analysis showed that patients diagnosed with HR-HPV variants had greater reductions in total motility and progressive motility than those diagnosed with LR-HPV.

Previous studies also described the negative impact of HPV infection on sperm count and semen volume^{6, 7}. However, our study did not observe these effects on patients' sperm count, contradicting these reports, which associated the HPV infection and the high-risk genotypes with lower sperm count. The impact of HPV on sperm count deserves further investigation, as additional factors not accounted for in this analysis could play significant roles in HPV-induced lower sperm count, such as the duration of infection, viral load, and individual immune responses^{2, 9}.

Our patients also presented with reduced normal morphology, consistent with prior reports describing a higher rate of teratozoospermia in patients with HPV^{5, 6, 9}. Earlier studies described the localization of HPV viral DNA in the head, midpiece, and tail of sperm, which was associated with a higher prevalence of abnormal morphology^{9, 23}. Additional studies attributed the abnormal sperm morphology to the effect of oxidative stress in semen as a result of the viral infection^{10, 24}. Indeed, this study observed a significant negative correlation between normal morphology and oxidative stress markers in the semen of the HPV-positive group. Other studies related the impact on sperm morphology to the effects of HR-HPV genotypes, which, in such cases, were found to generate more serious oxidative damage than LR-HPV⁹. Interestingly, however, the current analysis did not identify a significant effect of the HPV genotype on sperm

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morphology, suggesting that sperm morphological alterations could primarily be driven by HPV infection and oxidative stress, and not related to HPV genotype.

Oxidative stress markers (ROS, 8-OHdG, and MDA) were found to be elevated in the semen of HPV-positive individuals compared to the control group. Similar observations were described by several studies, which also reported increased oxidative stress levels among HPV-infected men^{1, 10}. Our analysis also showed that these elevated seminal oxidative stress markers were inversely correlated with key sperm parameters, including lower motility, reduced progressive motility, and increased abnormal morphology. Studies linked higher ROS levels in semen to excessive lipid peroxidation, DNA fragmentation, and protein denaturation^{25, 26}. Higher levels of 8-OHdG were also reported to cause sperm apoptosis, impaired chromatin compaction, and abnormal morphology^{24, 27}, while elevated MDA indicates excessive lipid peroxidation and deterioration in sperm quality²⁸. Semen from patients with HR-HPV genotypes was reported to have higher oxidative stress levels than LR-HPV^{1, 2}. However, our study found that the impact of HPV genotypes on levels of oxidative stress markers was mainly reflected in ROS levels, contradicting previous findings demonstrating a relationship between HPV genotype and semen levels of 8-OHdG^{1, 2, 10}.

Although the present study did not investigate whether patients of the study group exhibited signs of infertility, their semen characteristics were found below the reference values established by the WHO fertility guidelines¹⁴. These findings indicate a potential impairment in reproductive function among our study population, requiring further investigations to assess the long-term consequences of HPV infection on male fertility.

Study limitations included the selection of patients being conducted from a single health center, where a multicenter study approach can provide further details regarding the relationship between HPV infection and semen characteristics. The study also did not perform follow-up assessments on sperm parameters before and after HPV treatment, which could provide insight into the role of HPV treatment in improving semen characteristics. Further longitudinal studies could essentially enhance our understanding of the impact of the HPV infection on male fertility.

CONCLUSION

The current study demonstrated a significant association between HPV infection, lower semen quality, and higher levels of oxidative stress markers. Seminal levels of oxidative stress markers were inversely correlated with normal sperm parameters, supporting the role of HPV infection in male infertility. Additionally, the study highlights the importance of HPV early detection and screening, which could help reduce the adverse effects of HPV infection on male reproductive health.

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AUTHOR CONTRIBUTION

Hachem A: Carried out the design and implementation of the study, data collection and statistical analysis, writing of the manuscript, revision and approval of the final version.

Al-Shouk A: Carried out the study design, patients' selection and samples collection, writing of the manuscript, revision and approving the final version.

Hachim S: Data collection, preparing the first draft, revision and approving the final version.

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