Journal of Scientific Research and Reports

Volume 30, Issue 10, Page 947-959, 2024; Article no.JSRR.120352
ISSN: 2320-0227

Effect of Oxidative Stress on Sperm TERRA Expression and Chromatin Function in Infertile Males: A Preliminary Finding

Ankur [a], Mona Sharma [b++*], Ashutosh Halder [a#], Rima Dada [c†], Reeta Mahey [d†] and Neeraj Kumar [a‡]

[a] Department of Reproductive Biology, AIIMS, New Delhi, India.
[b] Department of Reproductive Biology, Room no. 2087, Teaching Blk, AIIMS, New Delhi, India.
[c] Department of Anatomy, AIIMS, New Delhi, India.
[d] Department of Obstetrics & Gynecology, AIIMS, New Delhi, India.

Authors' contributions

This work was carried out in collaboration among all authors. Author MS conceptualized the research work. Author MS designed the research work. Author Ankur performed the experimental work. Authors Ankur and MS did data analysis and result interpretation. Author Ankur wrote the Manuscript. Authors AH, RD and NK provided laboratory assistance. Author RM selected the patients for the study. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/jsrr/2024/v30i102516

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/120352

Original Research Article

Received: 01/06/2024
Accepted: 02/08/2024
Published: 19/10/2024

[++] Additional Professor;
[#] Professor& Head;
[†] Professor;
[‡] Scientist;
**Corresponding author: E-mail: monasharma@aiims.edu;*

Cite as: *Ankur, Mona Sharma, Ashutosh Halder, Rima Dada, Reeta Mahey, and Neeraj Kumar. 2024. "Effect of Oxidative Stress on Sperm TERRA Expression and Chromatin Function in Infertile Males: A Preliminary Finding". Journal of Scientific Research and Reports 30 (10):947-59. https://doi.org/10.9734/jsrr/2024/v30i102516.*

ABSTRACT

Telomeres are vital for cellular function. Oxidative stress is detrimental to telomere function as it causes telomere attrition. Increase in oxidative stress has a negative impact on sperm telomere and chromatin integrity and therefore, on sperm function and male fertility. TERRA, a long non-coding RNA is a fundamental component required for telomere length homeostasis.

Aim: The present work aimed to study the effect of H_2O_2 induced oxidative stress on sperm TERRA expression and chromatin function.

Methodology: 5 infertile and 5 fertile males were selected for the study. Baseline semen analysis was done as per WHO manual 2021. Sperm TERRA expression was assessed by real time qPCR. To assess chromatin function nuclear chromatin decondensation test was done.

Results: Study showed that 500µM H_2O_2 increased TERRA expression as compared to untreated groups and the difference was found to be statistically significant. As compared to controls, cases showed more fold increase in TERRA expression. When treated samples of control and cases were compared, cases had more TERRA expression than controls however, the baseline TERRA expressions in untreated samples were less in cases as compared to controls. Both comparisons yielded statistically nonsignificant results. When nuclear chromatin decondensation was compared before and after H_2O_2 treatment, the percentage of sperm head decondensation decreased in both cases and controls. When treated and untreated samples of cases and controls were compared, the difference was statistically nonsignificant.

Conclusion: Oxidative stress led to an increase in the expression of sperm TERRA and had a negative impact on nuclear chromatin decondensation. Similar studies with greater sample size can provide much more insight into TERRA mediated regulation of telomere function and chromatin integrity and how it is affected by oxidative stress.

Keywords: Oxidative stress; TERRA; chromatin integrity.

1. INTRODUCTION

Telomeres are 5’ TTAGGG 3’ tandem repeats located at the end of the chromosomes. They are a complex of DNA and proteins associated with RNA where protein and RNA contribute to the homeostasis and protection of telomeres. Telomeres provide the cells with a limited life span as telomeric repeats are lost with every cell replication due to end replication problem. Hence, telomeres act as biological clocks and regulate cell ageing. As the cell divides the telomere length keeps shortening [1].

Reactive oxygen species (ROS) are subproducts of cellular respiration. These are unstable oxygen containing molecules and primarily include O2−, H_2O_2, and OH•. Mitochondria is one of the major sources of ROS production [2]. When production of ROS exceeds the cell’s ability to neutralize them with antioxidants it leads to oxidative stress which can be detrimental to DNA, RNA as well as protein. When DNA gets oxidized there is an addition of oxygen to the nitrogenous bases which causes alteration in their structure making the DNA unstable and more susceptible to double and single stranded breaks. Out of the four nitrogenous bases, Guanine is most susceptible to oxidation. Telomeres are guanine rich structures which make them more prone to oxidative damage. Oxidative stress is known to be elevated in semen samples of infertile males and is a major contributor to DNA damage and telomere attrition [3]. Increased ROS concentrations and lower antioxidant levels in seminal plasma of infertile males were common findings as also reported in recent study [4]. Sperm and seminal plasma quality gets altered even in surgical conditions [5]. Spermatozoa with damaged or degraded DNA are linked to various conditions like poor embryonic development, poor fertilization rates and birth defects [6]. Sperm has highly compact nucleus still it is prone for oxidative damage [2]. Sperm mid piece contains mitochondria that generates ROS and is relatively deficient in antioxidative defense system [7]. Oxidative stress is also the cause of decreased fertilization potential of cryopreserved sperms [8-10].

Telomeres are heterochromatin structures hence they were thought to be transcriptionally silent, but it was recently discovered that they are transcribed by enzyme RNA polymerase II into telomeric repeat containing RNA also known as TERRA. TERRA is not translated into protein; it is a single stranded long noncoding RNA (lncRNA). TERRA originates from the transcription of C rich DNA strand hence it is G rich containing 5′- UUAGGG-3′ repeats. Recent

work suggests that TERRA sustains several important functions. Long noncoding RNA TERRA plays a crucial role in maintenance of telomere homeostasis [11]. Almost 7% of TERRA is polyadenylated at 3' and most of it is methylated at 5' end. TERRA is localized at the chromosome cap and forms DNA RNA hybrid structures called R loops by base pairing with one of the DNA strands and displacing the other to form a loop which are involved in telomere length shortening by inhibiting complete telomere replication. In cells where telomerase activity is absent TERRA increases in telomere length by homologous recombination [12]. Excessive TERRA expression can also lead to stalled replication fork and disruption of replication leading to telomere degradation. Long telomeres have high TERRA expression localized near the 3' overhang blocking the telomerase while short telomeres show low TERRA expression hence 3' end remains accessible for telomerase activity [13]. The availability of the 3' end of the chromosome for telomerase enzyme is essential for telomere lengthening as it acts as a substrate for telomerase enzyme. Excessive telomerase activity at the telomeric ends can lead to elongation of the chromosomes, stabilizing the telomere length hence cells avoiding aging and senescence. The oxidative stress caused by increased ROS production causes telomere attrition and DNA damage. ROS levels are elevated in sperms of infertile patients and contributes to male infertility.

TERRA regulates sperm telomere integrity and telomeres are considered regulators of sperm chromatin. Sperm chromatin decondensation is a prerequisite for male pronuclear formation. By evaluating the effect of oxidative stress on TERRA expression and nuclear decondensation, a functional association can be established between TERRA and chromatin function.

2. MATERIALS AND METHODS

As it was a preliminary study, total of 5 infertile and 5 fertile males were enrolled. The cases were selected from Obstetrics and Gynecology department of All India Institute of Medical Science, New Delhi. Both cases and controls were 25-40 years of age. Cases were apparent idiopathic infertile male showing at least one subnormal semen parameter. Controls were normozoospermic male showing at least one live birth. Cases with any known cause of infertility such as genital infection, endocrinological disorders, genital tract obstruction, varicocele, anti-sperm antibody, and chromosomal aneuploidy were excluded from the study. The protocol for this study was approved by the Institute's ethical committee **(**IECPG-209/24.03.2022, OT-07/22.12.2022) and written informed consent was taken from all the infertile cases and fertile controls.

Semen sample was collected after 2-7 days of abstinence period and baseline semen analysis was performed as per WHO manual 2021 [14]. After semen analysis, the samples from cases as well as controls were treated with 500µM H_2O_2 to induce oxidative stress. The concentration was chosen after preliminary experiments which showed 500µM H_2O_2 to produce maximum ROS levels. ROS levels were measured in relative light units/second (RLU/Sec) with chemiluminescence assay after adding luminol to the sample.

Sperm Preparation (By Swim Up): After complete liquefaction,1ml of semen was placed in a conical tube and centrifuged at 400g for 10 minutes. Post centrifugation, the supernatant was carefully discarded and the pellet layered with 1.5 ml fresh sperm rinse medium (Vitrolife). The tube was the incubated at an angle of 45° at 37°C for 1 hour time. The upper layer rich in motile sperm cells was collected carefully without disturbing the pellet. After extracting the upper layer containing motile cells, the media was divided into two aliquots and a treatment of 500µM H_2O_2 was given for 1 hour in one aliquot while one aliquot was left untreated.

Chemiluminescent assay: The chemiluminescence method is most frequently used as a direct approach for determining the production of ROS by spermatozoa. This assay was conducted to determine basal semen ROS levels and ROS levels of semen on exposure to 500µM concentration of H_2O_2 as this concentration giving maximum ROS was taken as a standard for further experiments.

The semen sample upon liquefaction was processed for ROS measurement. Aliquots of liquefied semen were prepared in centrifuge tubes of 1.5 ml. The first tube with no H_2O_2 treatment (0µM) was used to assess basal semen ROS levels. The remaining tubes with liquified semen were subjected to H_2O_2 500µM. The tubes were incubated at 37℃ for 1 hour. A 5mM stock of luminol (5-amino-2,3,-dihydro-1,4-phthalazinedione) in DMSO was prepared as a working solution and ten microliters were added to each tube and served as the probe. The luminometer measured chemiluminescence per

second for 10 min after addition of luminol and values of ROS were recorded for each sample.

Nuclear chromatin decondensation test: The nuclear chromatin decondensation test (NCDT) was used to assess the spermatozoa's capacity to decondense during fertilization. The test was carried out using the nuclear chromatin decondensation test kit (Hi-Tech Solutions). All the reagents were kept at 37°C for at least 30 minutes before the start of the experiment. 100µl of liquefied semen was taken in each of the two centrifuge tubes. The second tube was subjected to H_2O_2 treatment of 500µM concentration and both aliquots were incubated at 37℃ for 1 hour. 1 ml of wash buffer (provided in the kit) was added to the tubes, mixed well, and centrifuged for 5 min at 1500 rpm. After centrifugation, the supernatant was carefully discarded without disturbing the pellet. 200µl of NCD solution (provided in the kit) was added to the pellet and mixed well. The tubes were incubated at 37°C for 30 min. 50µl of stop solution (provided in the kit) was added to the tubes and incubated again at 37°C for 5 min. A drop of mixture solution was transferred onto the glass slide and examined under the microscope at 40x magnification. A minimum number of 200 condensed and decondensed heads were counted. Normal percentage for decondensed spermatozoa was > 70%.

TERRA expression: After sperm preparation using swim up method, sperm RNA was isolated using Qiagen RNeasy kit following the manufacturer's protocol. Total RNA was converted into cDNA. qPCR was done to check the relative fold change in TERRA expression. Cycles for RT qPCR was 95°C for 15 s, 60°C for 1 min for 40 cycles. Relative quantities of TERRA were calculated as the ratio of TERRA to GADPH (control) as (ΔCt = Ct TERRA − Ct GADPH) and $-2^{\Delta\Delta ct}$ assay was done to assess the fold change. TERRA and GAPDH specific primers were used for cDNA synthesis as well as qPCR (Tables 1-4).

RNA isolation: RNA extraction was done after sperm preparation using Qiagen RNeasy kit following the manufacturer's protocol. Approximately 10 million cells were pelleted down, the media was carefully removed and 600µl of RLT lysis buffer was added to the pellet. The solution was vortexed for 1 minute and 600µl of 70% ethanol was added in the tube. The solution was mixed well by pipetting. Up to 700µl of the sample, including any precipitate was transferred, to a RNeasy spin column placed in a collection tube. The column along with the collection tube was centrifuged for 15s at ≥8000g. The flow through was discarded. 700µl Buffer RW1 was added to the spin column and centrifuged for 15 s at ≥8000g to wash the spin column membrane. The flow through was discarded. 500 µl Buffer RPE was added to the spin column and centrifuged for 15s at ≥8000g to wash the spin column membrane. The flow through was discarded. 500µl Buffer RPE was again added to the spin column and the column along with the collection tube was centrifuged for 2 min at ≥8000g. The spin column was placed in a new collection tube and centrifuged at maximum speed for 1 minute. The Spin column was placed in a new 1.5 ml collection tube. 30µl of RNAse free water was directly added to the spin column membrane and centrifuged for 1min at ≥8000g. The concentration and purity of the eluted RNA was checked with the help of a nanodrop spectrophotometer. The RNA was stored at -80°C.

cDNA Synthesis: Total RNA was reverse transcribed into gene specific complementary-DNA (cDNA). Reverse transcription (RT) reaction was performed by using the Super Script™ III First-Strand Synthesis System kit (Invitrogen) according to the manufacturer's protocol. 280ng of total RNA was converted into cDNA. A reaction mixture of 12µl was prepared and incubated for 5 minutes at 65°C (Table 1).

An extension mixture was prepared and 8µl per tube was added to make the volume up to 20µl (Table 2). The 20 µl solution was then incubated at 55°C for 60 minutes followed by enzyme inactivation at 70°C for 15 minutes. The cDNA was stored at -20°C.

Table 1. Showing components for reaction mixture

Component	Amount	Finalconcentration
Upto280ngtotal RNA	As calculated	
dNTP mix	1µl	10mM
TERRA specific Primer	1µl	2µM
GAPDH specific Primers	1µl	2µM
Nuclease free water	As calculated	
Total	12µl	

Table 2. Showing components of extension mixture

Component	volume
Buffer	4µl
DTT	1µl
RNase OUT	2µl
SuperScript reverse transcriptase	1µl
Total	8µl

Table 3. Showing components for qpcr

Components	volume	concentration
cDNA	2µl	
Primers (forward + Reverse)	1µl	500nm
Sybr select Master mix	5µl	
Nuclease free water	2µl	
Total	10µl	

Table 4. Primer sequence of TERRA and GAPDH

Primer Sequence	Company
TERRA primers Forward- 5'GAGATTCTCCCAAGGC AAGG 3' Reverse- 5'ACATGAGGAATGTGGG TGTTAT 3'	IDT
GAPDH primers Forward- 5'AGCCACATCGCTCAGA CAC 3' Reverse- 5'GCCCAATACGACCAAA TCC 3'	IDT

Qpcr: The above prepared cDNA was used for qPCR (Table 3). Cycles for RT qPCR: 95°C for 15s, 60°C for 1 min for 40 cycles. Relative quantities of TERRA were calculated as the ratio of TERRA to GADPH (control) as (ΔCt = Ct TERRA − Ct GADPH). -2ΔΔct assay was done to assess the fold change (Table 4).

Statistical Analysis: Data was analyzed using MS Excel and GraphPad Prism 9.5.1 with student t test and p value of < 0.05 was considered statistically significant.

3. RESULTS

5 infertile cases and 5 fertile controls were recruited, and routine semen analysis was performed. Results of semen analysis are shown in Tables 5 and 6. Mean motility, vitality and morphology were higher and statistically significant in controls as compared to cases (Table 7). After semen analysis, the samples from cases as well as controls were treated with 500µM H_2O_2 to induce oxidative stress. ROS was measured with chemiluminescence assay after adding luminol to the sample. ROS levels were measured in relative light units/second (RLU/Sec). There was no significant difference observed in ROS levels between cases and controls in both treated and untreated experiments (Figs. 1 and 2).

To study TERRA expression, real time polymerase chain reaction was done after cDNA synthesis. Relative expression of TERRA between patient and control was assessed by -$2^{\Delta\Delta ct}$ method using a reference gene. It was observed that TERRA expression was increased after treatment in both cases and control after 1 hour incubation with 500µM H_2O_2 (Fig. 3).

In treated samples of cases and controls, TERRA expression was seen to be higher in cases as compared to controls. The difference was statistically non-significant. In untreated samples of cases and controls, cases had less TERRA expression as compared to controls. The difference was statistically non-significant (Figs. 4 and 5).

After treatment with 500µM H_2O_2, it was seen that sperm head decondensation was reduced in both cases and controls. The reduction in both cases and controls after treatment was statistically significant. When the sperm head decondensation was compared between untreated samples of cases and controls and between treated samples of cases and controls, the difference was seen to be minimal, and both the comparisons were statistically non-significant (photomicrograph 1; Tables 8-10; Figs. 6-8).

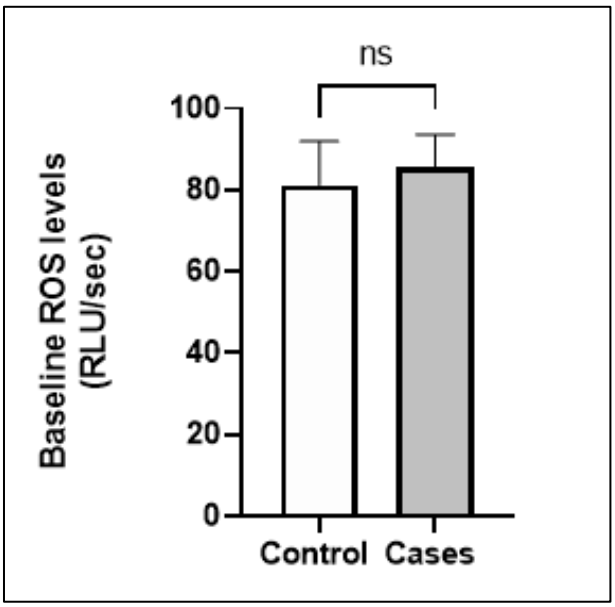


Fig. 1. Baseline ROS levels (Untreated)

Data analysis was done using student's t-test. Statistical graph was generated using graph pad prism software. p = 0.73; ns = nonsignificant.

Table 5. Semen parameters of cases

	P1	P2	P3	P4	P5
Age	38	32	27	33	28
pH	8.0	8.0	8.0	8.0	8.0
Volume(ml)	1.2	3	2.6	2.7	3.2
Motility	42%	43%	54%	58%	49%
Concentration (million/ml)	140	82	43	39	34
Vitality	49%	52%	63%	60%	57%
Morphology	1%	1%	1%	1%	2%

Table 6. Semen parameters of controls

	C1	C2	C3	C4	C5
Age	33	37	35	35	38
pH	8.0	8.0	8.0	8.0	8.0
Volume(ml)	2	2.5	3.5	2.5	3
Motility	70%	68%	62%	65%	59%
Concentration (millionperml)	132	70	23	70	30%
Vitality	84%	81%	68%	72%	65%
Morphology	9%	7%	6%	8%	8%

Table 7. Comparison of Semen parameters (Mean ± SD) between Cases and Controls

	Concentration (million/ml)	Motility	Vitality	Morphology
Cases (n=5)	67.6±44.72	49%±0.069	56%±0.057	1%±0.004
Controls (n=5)	65±44.38	74%±0.082	74%±0.082	8%±0.011
Pvalue	0.92	<0.001	0.004	<0.001

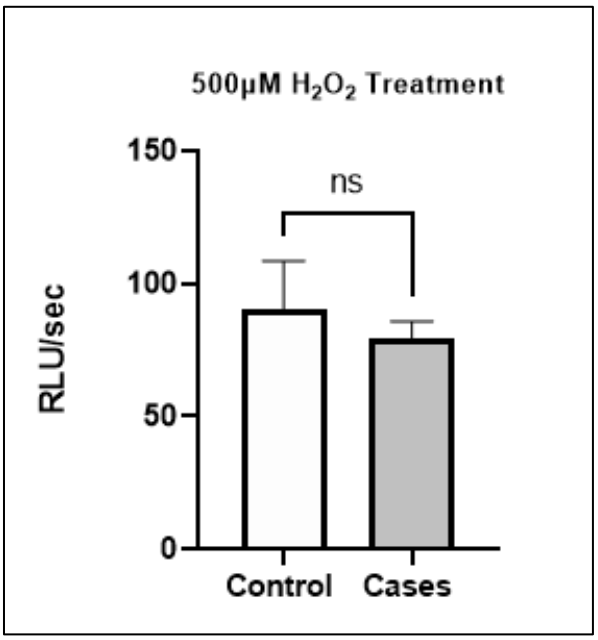


Fig. 2. Mean ROS levels after 500µM H_2O_2 treatment

Data analysis was done using student's t-test. Statistical graph was generated using graph pad prism software p = 0.52

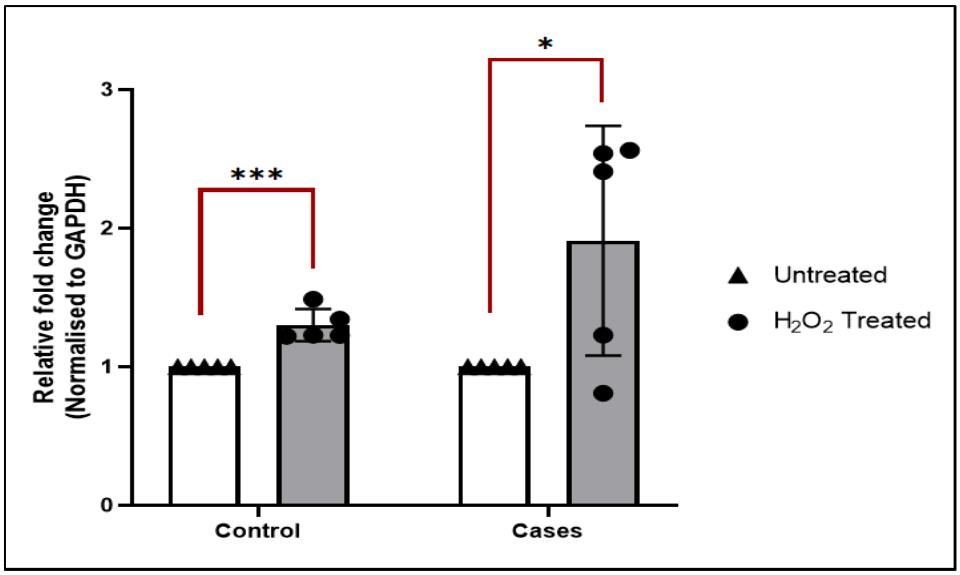


Fig. 3. Relative fold change in TERRA mRNA levels before and after H_2O_2 treatment within the Controls and Cases. Normalized to GAPDH.

Data analysis was done using student's t-test. Statistical graph was generated using graph pad prism software. P (control) < 0.001, P (cases) = 0.04

Table 8. Sperm nuclear decondensation of controls before and after H_2O_2treatment

Nuclear decondensation (%)	C1	C2	C3	C4	C5
Untreated group	78	82	85	86	81
Treated group	68	76	80	75	78

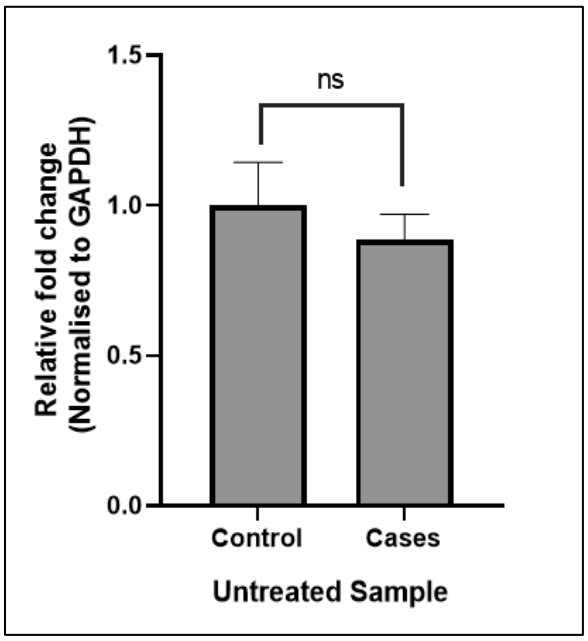


Fig. 4. Relative fold change in TERRA mRNA levels in Controls and Cases (Without treatment).
Normalized to GAPDH. Data analysis was done using student's t-test. Statistical graph was generated using graph pad prism software. P = 0.5; ns = non-significant

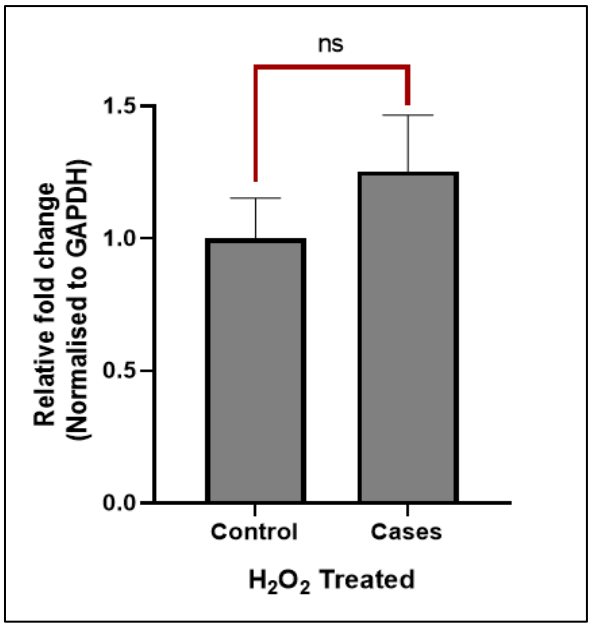
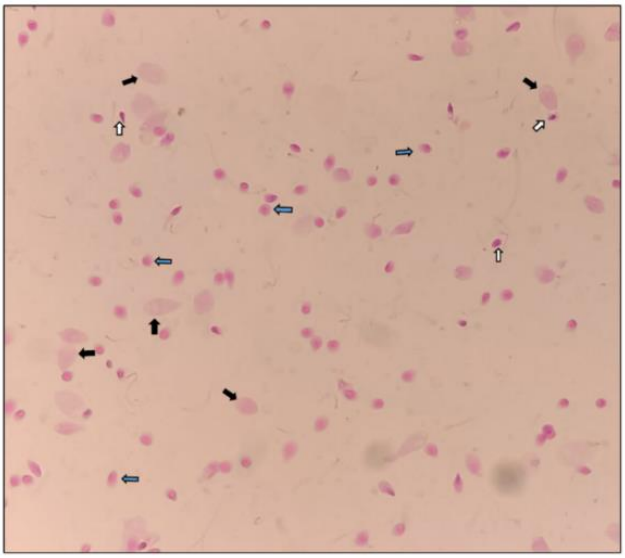


Fig. 5. Relative fold change in TERRA mRNA levels in controls and cases after H_2O_2 treatment
Normalized to GAPDH. Data analysis was done using student's t-test. Statistical graph was generated using graph pad prism software. P = 0.3; ns = non-significant

Table 9. Sperm nuclear decondensation in cases before and after H_2O_2treatment

Nuclear decondensation (%)	P1	P2	P3	P4	P5
Untreated group	84	79	80	84	85
Treated group	74	66	75	65	78



Picture 1. sperm heads marked with white arrows, moderately decondensed heads marked with blue arrows and grossly decondensed sperm heads marked with black arrows

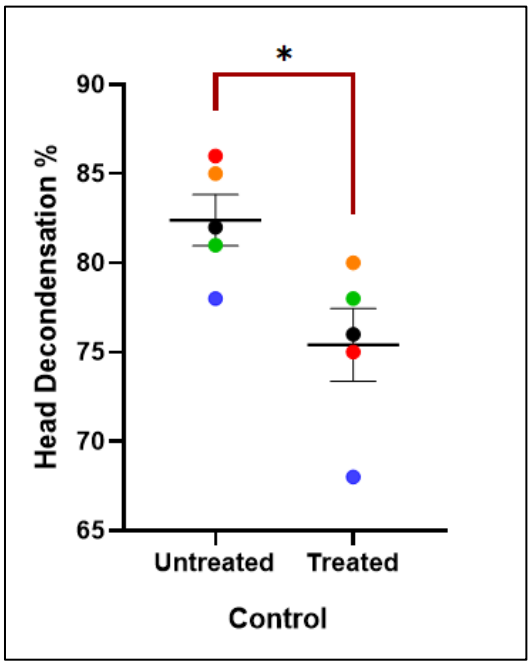


Fig. 6. Nuclear decondensation before and after H_2O_2 treatment in Controls
Data analysis was done using student's t-test. Statistical graph was generated using graph pad prism software. p = 0.02

Table 10. Comparison of sperm nuclear decondensation (mean ± SD) between cases vs controls (treated and untreated samples)

	Untreated	Treated	p value
Cases	82% ± 0.02	72% ± 0.05	0.005
Control	82% ± 0.03	75% ± 0.04	0.02
p value	0.99	0.22	

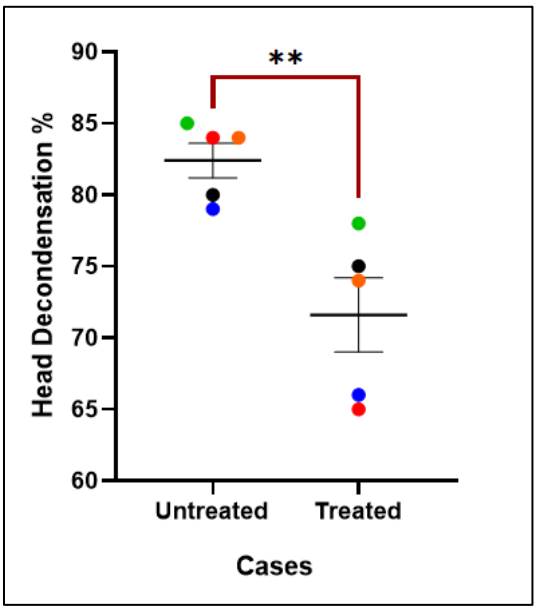


Fig. 7. Nuclear decondensation before and after H_2O_2 treatment in Cases

Data analysis was done using student's t-test. Statistical graph was generated using graph pad prism software. p = 0.005

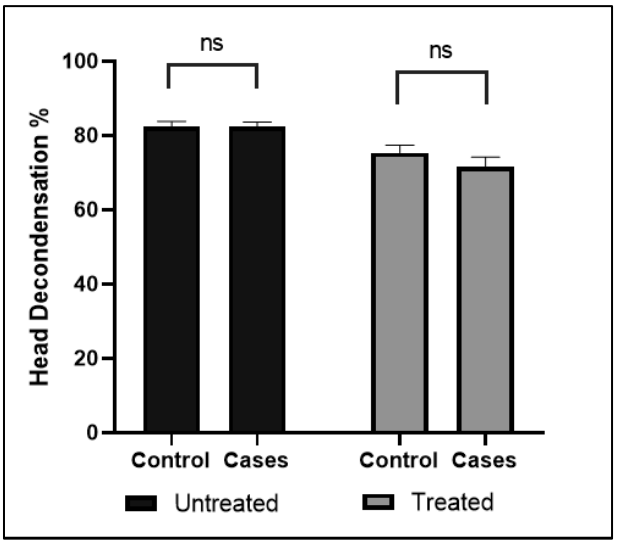


Fig. 8. Nuclear decondensation before and after H_2O_2 treatment in Controls and Cases (treated and untreated samples)

Data analysis was done using student's t-test. Statistical graph was generated using graph pad prism software. Untreated p value = 0.99, Treated p value = 0.22

4. DISCUSSION

Oxidative stress is a major etiologic factor in numerous human diseases, including cardiovascular disorders, cancers, aging and infertility. There have been studies on oxidative stress upregulating expression of genes regulating antioxidative defense. One example is SKN-1 gene which is best known as a regulator of antioxidant defense [15]. Other studies report cross-talk between non-coding RNAs and oxidative stress in various disorders such as Parkinson's diseases [16]. In present study, oxidative stress was induced by treating semen samples with 500µM concentration of H_2O_2 after few preliminary experiments that showed maximum ROS produced by 500µM H_2O_2. The baseline ROS levels were higher in cases as

compared to controls though the difference was not significant. This finding indicates that the effect of H_2O_2 treatment produced a similar extent of oxidative stress in cases and controls. Studies have shown the effect of 500µM H_2O_2 treatment on sperm and the embryo quality that it yields [17]. The effect of 500µM H_2O_2 is also seen on sperm capacitation and acrosomal reaction [18]. After treatment with 500µM H_2O_2, sperm RNA was isolated to assess the effect of treatment on TERRA expression. It was seen that after the treatment, TERRA expression increased in both cases and controls. Controls showed a 0.3 folds increase when compared to untreated samples while cases showed a 0.9 folds increase when compared to baseline untreated samples. 500µM H_2O_2 treatment is also seen to be increasing the TERRA expression in somatic cells as a protective response towards the telomeres [19]. The antioxidant defense in semen sample of infertile males is also seen to be poor as compared to fertile males [20]. The reason for more TERRA expression in cases as compared to controls could be that the antioxidant capacity in fertile males is higher that protected the telomeres from being affected by the oxidative stress. In infertile cases this antioxidant capacity was compromised leading to telomeres getting affected by oxidative stress hence, an elevated TERRA expression. Transcription is known to be repressed in sperm due to chromatin condensation, but these cells still have transcription potential as the telomeric ends are not fully condensed [21]. Chromatin in mature sperms is seen to be associated with various transcription factors and promotor sequences [22]. Effect of oxidative stress has also been studied in another regulator of telomere which is TRF1 (telomeric repeat-binding factor 1). Shelterin complex, a specialized complex of six proteins consists of TRF1, TRF2 (telomeric repeat-binding factor 2), POT1 (Protection of telomere 1), TIN2 (TRF1-interacting nuclear factor 2), TPP1 (TIN2 and POT1-interacting nuclear protein 1), and RAP1 (repressor and activator protein 1). Treatment with H_2O_2 altered the expression of TRF1 in both fertile and infertile males [23]. Despite of sperms being transcriptionally inert, studies have shown that factors like cryopreservation and treatment with fluoride, which is a pollutant can induce effect on their gene expression.

After treatment with fluoride, both repression and induction of several genes was seen at transcription level in mice sperms [24]. Both cryopreservation and fluoride are known to induce oxidative stress in cells [25]. Exposure to H_2O_2 and induction of oxidative stress can act as a stimulus and induce the cell to increase TERRA expression to protect the telomeres in sperm. When comparison was made between the untreated samples of cases and controls, TERRA expression was seen to be slightly more in controls as compared to cases, but the difference was not statistically significant. When treated control and cases samples were compared, cases showed more TERRA expression than controls, but the difference was not statistically significant.

Nuclear decondensation was decreased in both cases and controls after treatment. A drop of about 10% in head decondensation percentage was seen in cases where the average decondensation percentage before treatment was 82% and after treatment it was 72%. In controls nuclear decondensation was reduced by 7% where the average decondensation percentage before treatment was 82% and after treatment it was 75%. Both the comparisons showed a statistically significant difference between treated and untreated samples of cases and controls.

When untreated samples of cases and controls were compared, the difference was minimal showing both cases and controls had almost same nuclear decondensation ability. Case group had a lower mean motility, vitality and morphology despite of that, the group showed equivalent decondensation as fertile control groups. It can be inferred that whatever the cause of their infertility was, it was not affecting the chromatin packaging and head decondensation ability of the sperms. A comparison between treated samples of cases and controls was also done which showed that cases had slightly less head decondensation percentage as compared to controls, but the difference was non-significant. After treatment it was seen in both cases as well as controls that the TERRA expression went up whereas the nuclear chromatin decondensation percentage went down. Compensatory rise in TERRA expression and lower nuclear chromatin decondensation were the result of oxidative stress.

5. CONCLUSION

Telomeres are prone to damage by oxidative stress leading to telomere attrition. Increase in oxidative stress has a negative effect on male

fertility and sperm health. This study was focused on the effect of oxidative stress on TERRA which is a fundamental component required for telomere homeostasis. Induced oxidative stress increased a compensatory rise in TERRA expression whereas it decreased extent of sperm nuclear decondensation. Therefore, it can be suggested that oxidative stress commonly seen in infertile males affects chromatin integrity that may be associated with failed fertilization outcome seen in these patients. But whether rise in TERRA is beneficial or detrimental to sperm telomeres needs further studies to be conducted in larger cohorts.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Valdes AM, Andrew T, Gardner JP, Kimura M, Oelsner E, Cherkas LF, et al. Obesity, cigarette smoking, and telomere length in women. The lancet. 2005;366(9486):662-664.
2. Murphy MP. How mitochondria produce reactive oxygen species. Biochemical Journal. 2009;417(1):1-13.
3. Aitken RJ and Clarkson JS. Cellular basis of defective sperm function and its association with the genesis of reactive oxygen species by human spermatozoa. Reproduction.1987;81(2):459-469.
4. DipankarSP, Kumar T, Begum HA, Naik BN, Kumar Y, Sharma M, et al. Semen Quality in Males Suffering From COVID-19: A Pilot Study. Cureus. 2022;14(11): e31776.
5. Asuri K, Mohammad A, Prajapati OM, Sagar R, Kumar A, Sharma M, et al. A prospective randomized comparison of sexual function and semen analysis following laproscopic totally extraperitoneal inguinal hernia repair. Surgical Endoscopy; 2020, Jun.
6. Sukcharoen N, Keith J, Irvine DS, Aitken RJ. Predicting the fertilizing potential of human sperm suspensions in vitro: importance of sperm morphology and leukocyte contamination. Fertility and sterility.1995;63(6):1293-1300.
7. Sharma M, Kumar A. The Sperm. In: Basics of Human Andrology, 1st ed. Kumar and Sharma, eds. Springer Nature, Singapore. 2017; 171-203.
8. Sharma Y, Sharma M. Sperm cryopreservation: Principles and Biology. J Infertil Reprod Biol. 2020;8(3):43-48.
9. Sharma Yashaswi, Sharma Mona. Biophysics of Cryopreservation. International Journal of Thermodynamics. 2022;25(1):17-27.
10. Sharma Y, Sharma M, Halder A, Mahey R, Kumar N, Dipankar S. Temporal analysis of cryopreservation effects on human sperm phospholipase C zeta expression profile: Laboratory preliminary findings. J Lab Physicians. 2024;16(3): 328-337.
11. Azzalin CM, Reichenbach P, Khoriauli L, Giulotto E. and Lingner J. Telomeric repeat–containing RNA and RNA surveillance factors at mammalian chromosome ends. Science. 2007; 318(5851):798-801.
12. Wang C, Zhao L, Lu S. Role of TERRA in the regulation of telomere length. International journal of biological sciences. 2015;11(3):316.
13. Cusanelli E and Chartrand P. Telomeric noncoding RNA: Telomeric repeat-containing RNA in telomere biology. Wiley Interdisciplinary Reviews: RNA. 2014;5(3):407-419.
14. WHO Laboratory Manual for the examination and processing of human semen. 6th ed; 2021.
15. Kaushik N, Rastogi S, Verma S, Pandey D, Halder A, Mukhopadhyay A, Kumar N. Transcriptome analysis of insulin signaling-associated transcription factors in C. elegans reveal their genome-wide target genes specificity and complexity. International Journal of Molecular Sciences. 2021;22(22):12462.
16. Zhang H, Liu X, Liu Y, Liu J, Gong X, Li G, Tang M. Crosstalk between regulatory non-coding RNAs and oxidative stress in Parkinson's disease. Front Aging Neurosci. 2022, Aug 9;14:975248.
17. Silva PF, Gadella BM, Colenbrander B and Roelen BA. Exposure of bovine sperm to pro-oxidants impairs the developmental competence of the embryo after the first cleavage. Theriogenology. 2007;67(3):609-619.

18. Rivlin J, Mendel J, Rubinstein S, Etkovitz N, Breitbart H. Role of hydrogen peroxide in sperm capacitation and acrosome reaction. Biol Reprod. 2004;70(2):518-22.
19. Galigniana NM, Charó NL, Uranga R, Cabanillas AM and Piwien-Pilipuk G. Oxidative stress induces transcription of telomeric repeat-containing RNA (TERRA) by engaging PKA signaling and cytoskeleton dynamics. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research. 2020;1867(4):118643.
20. Pahune PP, Choudhari AR, Muley PA. The total antioxidant power of semen and its correlation with the fertility potential of human male subjects. JCDR. 2013;7(6): 991-995.
21. Ren X, Chen X, Wang Z. and Wang D. Is transcription in sperm stationary or dynamic? Journal of Reproduction and Development. 2017;63(5):439-443.
22. Jung Y H, Sauria MEG, Lyu X, Cheema MS, Ausio J, Taylor J, Corces VG. Chromatin States in Mouse Sperm Correlate with Embryonic and Adult Regulatory Landscapes. Cell Reports. 2017;18(6):1366–1382.
23. Anisha J, Mona S, Rima D, Reeta M, Ashutosh H, Deepak P. Effect of oxidative stress on sperm shelterin TRF1 expression and 008 chromatin function in infertile males: Preliminary Findings. Glob J Reprod Med. 2024;10(5):555800.
24. Sun Z, Niu R, Wang B, Jiao Z, Wang J, Zhang J, et al. Fluoride-induced apoptosis and gene expression profiling in mice sperm *In vivo*. Archives of Toxicology. 2011;85:1441-1452.
25. Agarwal A. Saleh RA, Bedaiwy MA. Role of reactive oxygen species in the pathophysiology of human reproduction. Fertil Steril. 2003;79:829-843.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/120352