Iran Red Crescent Med J. 2019 July; 21(7):e86806. doi: 10.5812/ircmj.86806.
Published online 2019 July 21. Research Article

Role of Epididymis and Testis in Nuclear Factor Erythroid 2-Related Factor 2 Signaling in Mouse Experimental Cryptorchidism

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Received 2018 November 26; **Revised** 2019 June 02; **Accepted** 2019 June 03.

Abstract

Background: Cryptorchidism is a common gonadal disease in neonates that promotes oxidative stress and reactive oxygen spices production following the presence of testicles in ectopic places. Nuclear factor erythroid 2-related factor 2 (Nrf2) signaling could prevent oxidative stress damage through the Nrf2-driven antioxidants.
Objectives: The current study aimed at evaluating the pattern of gene expressions related to this pathway in testis and epididymis of mice experimentally subjected to cryptorchidism.
Methods: The current experimental study was conducted on 48 male mice randomly divided into six groups at Arak University of Medical Sciences, Arak, Iran, 2018. Cryptorchidism was induced in the left testis of mice in five experimental groups by removing the testis from the scrotum and suturing it to the muscles of the abdominal wall. One group remained intact as the control group. Sperm parameters were analyzed one day, as well as one, two, four, and eight weeks after the operation. The expression of Nrf2, NQO1, HO1, and Keap1 genes in testis and epididymis was assessed using quantitative reverse transcriptase- polymerase chain reaction.
Results: Sperm analysis showed a significant decrease in sperm number, viability, normal morphology (except in the one day group), and motility in all experimental groups compared to the control group ($P \leq 0.05$). The expression level of Nrf2 (in testis tissue), Keap1 (in both testis and epididymis), and HO1 (in testis tissue) showed no changes over the time ($P > 0.05$), while the expression of other genes decreased remarkably ($P \leq 0.05$) in both testis tissue and epididymis in a time-dependent manner.
Conclusions: The current study findings showed that the expression of genes involved in NRF2 systems decreased in the epididymis of cryptorchid mice more than testis and it suggested that treatment with Nrf2 inducer could decrease destructive effects of cryptorchidism on the reproductive system.

Keywords: Antioxidants, Cryptorchidism, Epididymis, Mice, NF-E2-Related Factor 2, Oxidative Stress, Reverse Transcriptase Polymerase Chain Reaction, Sperm Count, Testis

1. Background

All animals encounter reactive oxidants generated from cellular metabolism or environmental exposure in life (1, 2). In normal conditions, the production of reactive oxidants is under the control of some defense mechanisms such as antioxidant enzymes to minimize cell damage (3). Recent studies demonstrated that nuclear factor erythroid 2-related factor 2 (Nrf2) has an important role in upregulating antioxidant enzymes including γ-glutamylcysteine synthetase (GCS), hemeoxygenase 1 (HO-1), and NAD (P) H: quinone oxido-reductase-1 (NQO1) (2). Normally, Nrf2 binds to Kelch-like ECH-associated protein 1 (Keap1), which forms an inactive complex in the cytoplasm (4, 5). Oxidative stress conditions activate Nrf2 by releasing it from Keap1 (4). Activated Nrf2 is transferred into the nucleus and after binding to antioxidant response element (ARE), regulates the expression of genes involved in cell protection against oxidative injury (1).

Cryptorchidism, the absence of one or both testes from the scrotum, has a multifactorial etiology including endocrinal, environmental, anatomical, and mechanical factors (6). Following cryptorchidism, testicular hyperthermia induces germ cell apoptosis and reactivates oxygen

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species (ROS) production, which causes deleterious effects on testis tissue leading to male fertility (3). The testis has several heat-tolerance mechanisms to protect germ cells against oxidative stress, including GPX, HO-1, and NQO1 (2). The expression of HO1 and NQO1 controlled by Nrf2, increases within the first days after scrotal heating (7).

The components involved in the expression of Nrf2 signaling pathway vary between tissue and species. This detoxification mechanism plays a protective role in the reproductive system (8). It was observed that Nrf2 knockdown in male mice causes spermatogenesis disruption (9) and decreases the expression of Nrf2 reported in males with low sperm motility (10). However, a change in Nrf2 signaling in gonadal diseases is not well understood.

2. Objectives

To the authors' best knowledge, the current study was the first evidence of alteration in Nrf2/Keap1 following cryptorchidism. By understanding the changes in this pathway, suitable treatment strategies can be used to decline testis tissue damage caused by cryptorchidism.

3. Methods

3.1. Animals

Research and animal care protocols were approved by the Ethics Committee of Arak University of Medical Sciences (1395.317). In vivo experiments were performed at Arak University of Medical Sciences, Arak, Iran in 2018 on adult male NMRI mice (20 - 25 g, Pasteur, Iran). Animals were housed at 24°C under controlled conditions with free access to water and food.

3.2. Experimental Design

In the current experimental study, animals were randomly assigned to six groups (n = 8): one intact group as the control and five groups undergoing surgical procedures. Mice in groups undergoing surgery were anesthetized with intraperitoneal injection of 100 mg/kg ketamine and 10 mg/kg xylazine (both from Alfasan, Iran); then cryptorchidism was induced by pushing left testis via inguinal canal into the abdominal cavity followed by suturing testis to the abdominal wall, cutting gubernaculum, and closing the inguinal canal (11). Control and experimental animals were scarified one day, as well as one, two, four, and eight weeks after cryptorchidism induction.

Left testis and head of epididymis were removed and stored at -70°C for further experiments, and the left cauda epididymis was carefully removed and placed in 500 μL of Dulbecco's modified eagle medium (DMEM; Gibco, Germany) to analyze sperm parameters.

3.3. Analysis of Sperm Parameter: Motility, Count, Morphology, and Viability

The left cauda epididymis in DMEM was minced and incubated at 37°C in 5% CO_2 for 30 minutes. Next, one drop of sperm suspension was placed on a Neubauer chamber and covered by cover slide (12). Then, the percentage of motile sperm and sperm count (12) were evaluated under a light microscope (for the sperm motility evaluation, a X 400 magnification was used). The sperm count was expressed as $\times 10^6$/mL.

The sperm smears were stained by Papanicolaou staining technique and sperm morphology was evaluated under a light microscope (X 400 magnification). One hundred sperm from different fields were counted to determine the morphological abnormalities (13).

For sperm viability analysis, Eosin-Nigrosin (Merck, Germany) staining was used according to the standard protocol. In this technique, the dead sperms turn red, while live sperms remained unstained (14).

3.4. RNA Isolation and cDNA Synthesis

After sampling, the expression of Nrf2, Keap1, HO-1, and NQO1 genes as well as GAPDH (as internal control) in all groups was studied by quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR). Total RNA was extracted using peqGold RNA TriFast (PeqLab, Germany) according to the manufacturer's instructions. The RNA pellet was dissolved in diethylpyrocarbonate-treated water (DEPC treated water; SinaClon, Iran), and quantified using spectrophotometer at 260 nm wavelength. The integrity of the extracted total RNA was assessed by agarose gel electrophoresis and verified by the presence of the 28S and 18S rRNA bands. Immediately after RNA preparation, 2 μg of total RNA was used for cDNA synthesis in a total volume of 20 μL using RevertAid™ First Strand cDNA Synthesis Kit (Aryatous, Iran). The cDNA was stored at -70°C before use.

3.5. Quantitative Real-Time Polymerase Chain Reaction

The qRT-PCR was carried out using the LightCycler® RT-PCR (Roche, USA). The qRT-PCR was performed in a total volume of 20 μL containing 2 μL of cDNA (5-fold dilution), 0.5 μL of 5 mM/L solutions of each of the forward and reverse primers, and 10 μL of 2X SYBR green DNA PCR Master Mix (Yekta Tajhiz Azma, Iran). Primer sequences are listed in Table 1. Each sample was loaded in duplicate. Melt curve analysis was performed after each run to check for the presence of non-specific PCR products and primer dimers. The expression ratio was calculated using relative formula based on the comparative CT method ($^{\Delta\Delta}$CT).

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Table 1. The Sequences and Length of Primers Used to Amplify Nrf2 Signaling Pathway

Gene	Primer Sequences (5' to 3')	Product Length, bp
NQO1	Forward: CCA ATC AGC GTT CGG TAT T	184
	Reverse: AGT TCA TAG CAT AGA GGT CAG A	
Nrf2	Forward: CAC ATC CAG ACA GAC ACC AG	148
	Reverse: TAT CCA GGG CAA GCG ACT C	
Keap-1	Forward: GTG GGC GAG AAG TGT GTC C	224
	Reverse: AAG AAC TCC TCC TGC TTG G	
HO1	Forward: TCA CAG ATG GCG TCA CTT C	223
	Reverse: CAC ATT GGA CAG AGT TCA CA	
GAPDH	Forward: AGC AAG GAC ACT GAG CAA GAG	152
	Reverse: GCA GCG AAC TTT ATT GAT GGT	

3.6. Statistical Analysis

The results were expressed as mean ± standard error (SE). The statistical significance difference between the mean values of control and experimental groups in different intervals was analyzed by repeated measures analysis of variance (ANOVA) followed by a Bonferroni post hoc test; $P \leq 0.05$ was considered the level of significance.

4. Results

4.1. Analysis of Sperm Parameters After Cryptorchidism Induction

According to the current study results, the sperm count, viability, and progressive and non-progressive motility decreased significantly in all of the experimental groups compared to the control group. A considerable decrease was also observed in the normal morphology of all the experimental groups, except the one-day group. The number of immotile sperms increased notably in all experimental groups. None of the progressive and non-progressive sperms were detected eight weeks after cryptorchidism induction. Sperm parameters in all experimental groups are summarized in Table 2.

4.2. Expression of Nrf2 in the Testis and Epididymis of Cryptorchid Mice

The expression pattern of Nrf2 in the testis tissue of cryptorchid mice showed no obvious changes over the time ($P > 0.05$) (Figure 1A). The Nrf2 mRNA level dropped significantly ($P \leq 0.001$) in the epididymis of all experimental groups compared to the control group, except one day and eight weeks after operation (Figure 1B).

4.3. Expression of Keap1 in the Testis and Epididymis of Cryptorchid Mice

The expression pattern of Keap1 in the testis tissue and epididymis of cryptorchid mice was analyzed by repeated-measures ANOVA and showed no changes over time ($P > 0.05$) (Figure 2).

4.4. Expression of HO1 in the Testis and Epididymis of Cryptorchid Mice

The expression pattern of HO1 did not significantly ($P > 0.05$) change with time while in the epididymis of cryptorchid mice; variable response changed one and two weeks after induction in comparison with the control group (Figure 3).

4.5. Expression of NQO1 in the Testis and Epididymis of Cryptorchid Mice

The NQO1 mRNA pattern in testis tissue showed that after cryptorchidism induction, there was a significant reduction in gene expression eight weeks after operation compared to the control group (Figure 4A). In addition, the expression pattern of NQO1 changed two, four, and eight weeks after cryptorchidism induction compared to the control group (Figure 4B).

5. Discussion

In the present study, several gene expressions involved in the Nrf2 signaling pathway were investigated in the testis and epididymis of experimental model of cryptorchidism. For this purpose, cryptorchidism was induced in the left testis of mice by suturing the testis to the muscles of abdominal wall (15). Sperm quality parameters were analyzed at different intervals from surgery. The current study results showed that all sperm quality parameters, including sperm count, morphology (except in the one-day group), viability, and motility reduced in all experimental groups. Several studies demonstrated that cryptorchidism has deleterious effects on testis tissue and semen quality (16, 17). It is clear that low temperature is essential for normal spermatogenesis, and scrotum provides the necessary environment for testis function (3). During cryptorchidism, high temperature leads to oxidative stress and ROS production in testicular tissue located in the abdominal cavity or inguinal canal (3, 18). As a result of cryptorchidism, excessive ROS production, disturbance in the sexual hormone, and germ cells apoptosis are responsible for a reduction in sperm quality and fertility (3, 19, 20). In addition, the activity of antioxidant enzymes such as classical cellular glutathione peroxidase (GSHPX), Cu, Zn, superoxide dismutase (SOD), and catalase

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Table 2. Analysis of Sperm Quality Parameters in the Experimental and Control Groups[a]

	Count, $\times 10^6$	Viability, %	Morphology, %	Immotile Sperm, %	Non-Progressive Sperm, %	Progressive Sperm, %
Control	27800 ± 5946.84	76.80 ± 0.96	88.40 ± 1.60	39.60 ± 4.22	36.00 ± 3.56	24.40 ± 6.53
1 d	7300 ± 1570.03[c]	58.40 ± 1.53[c]	82.20 ± 2.41	74.00 ± 4.26[c]	17.80 ± 3.24[c]	8.20 ± 2.53[b]
1 w	4666.66 ± 1102.39[c]	33.11 ± 2.11[d]	40.77 ± 5.07[d]	94.55 ± 1.78[d]	5.00 ± 1.51[d]	0.44 ± 0.33[d]
2 w	2166.66 ± 1142.60[d]	14.22 ± 6.31[d]	12.22 ± 7.6[d]	98.12 ± 1.31[d]	1.55 ± 1.14[d]	0.11 ± 0.11[d]
4 w	1666.66 ± 986.01[d]	11.66 ± 5.63[d]	18.00 ± 8.93[d]	98.87 ± 1.12[d]	0.88 ± 0.88[d]	0.11 ± 0.11[d]
8 w	83.33 ± 56.18[d]	3.08 ± 3.08[d]	0.25 ± 0.25[d]	100 ± 1.23[d]	0.00 ± 0.00[d]	0.00 ± 0.00[d]

[a] Sperm quality decreased in all the experimental groups in comparison with the control group.
[b] $P \leq 0.05$
[c] $P \leq 0.01$
[d] $P \leq 0.001$

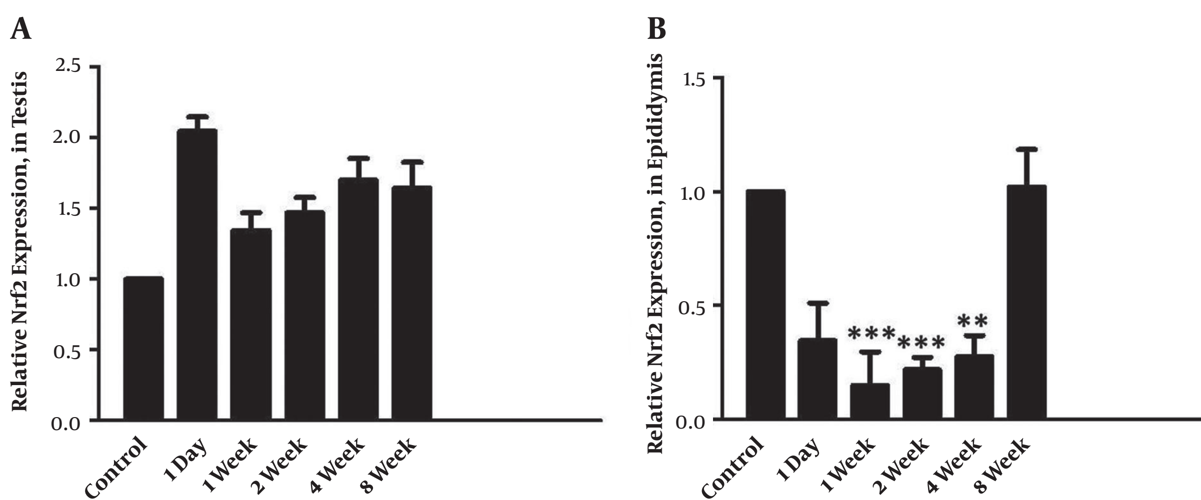

Figure 1. RT-PCR analyzed patterns of Nrf2 expression in the testis tissue (A) and epididymis (B) of cryptorchid mice at different intervals. ** $P \leq 0.01$, *** $P \leq 0.001$

drops in cryptorchidism (21). One of the cellular detoxification pathways involved in removing excessive ROS products through increasing the antioxidant levels is the Nrf2 signaling pathway (1, 2). The current study led towards enhancing the available understanding of the expression of genes related to Nrf2 signaling in the testis and especially in the epididymis of the cryptorchid mice. According to the current study results cryptorchidism could not induce Nrf2/Keap1 pathway in testis tissue, while in epididymis cryptorchidism it could down regulate this signaling pathway over time. These differences in Nrf2/Keap1 expression suggested that in epididymis, there was the most alteration in gene expressions involved in the Nrf2 signaling pathway and testicular heating occurred during cryptorchidism that had various effects on different parts of reproductive system. Wajda et al., previously explained that Nrf2 expression is higher in rat's corpus of epididymis than its liver, testis, and other parts of epididymis (8). Epididymis plays important roles in sperm maturation, motility, and storage (21). The activity of some detoxifying enzymes such as gamma-glutamyltranspeptidase (GGT) and glutathione S-transferase (GST) was detected in epithelial cell culture of human epididymis (22). In epididymis of male Nrf2-knockout mice, lipid peroxidation increased, and expression of GST, sod2, and enzymatic activities of glutathione reductase and glutathione peroxidase decreased (9). These results highlighted the protective role of epididymis against oxidative stress in the reproductive system. The current study data showed that the expression of Nrf2 decreased one, two, and four weeks after operation, and Keap1 expression also decreased, but not significantly. Also, the mRNA level of HO1 decreased one and two weeks after the induction of cryptorchidism, and NQO1 expression decreased two, four, and eight weeks after surgery. Nrf2 has a short half-life (around 20 minutes) (23) and is immediately degenerated by Keap1 (4). The whole body heat

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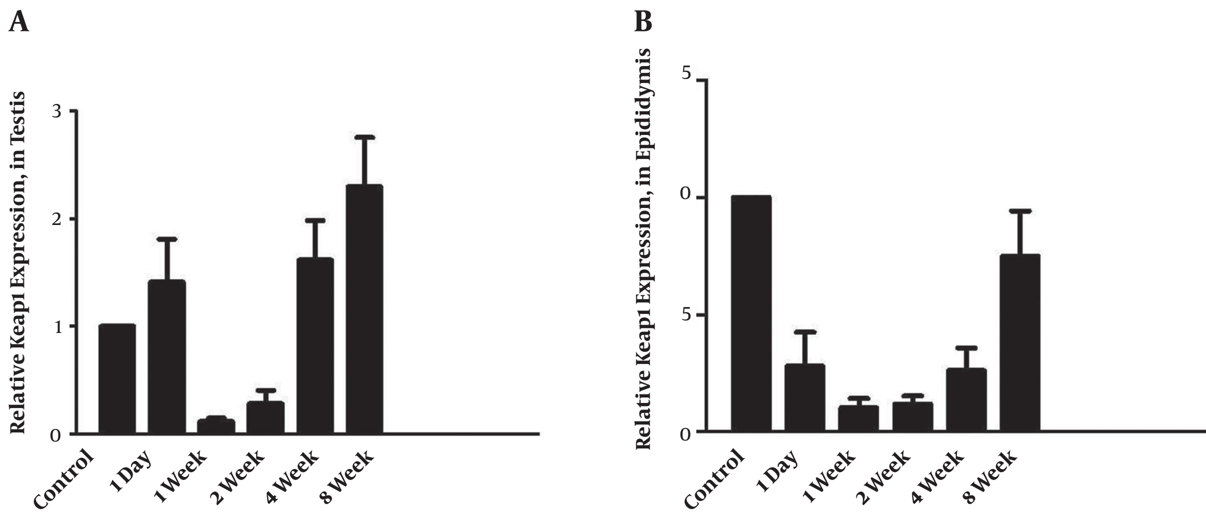

Figure 2. The expression level of Keap1 gene in the testis tissue (A) and epididymis (B) of cryptorchid mice determined by RT-PCR is presented in different groups.

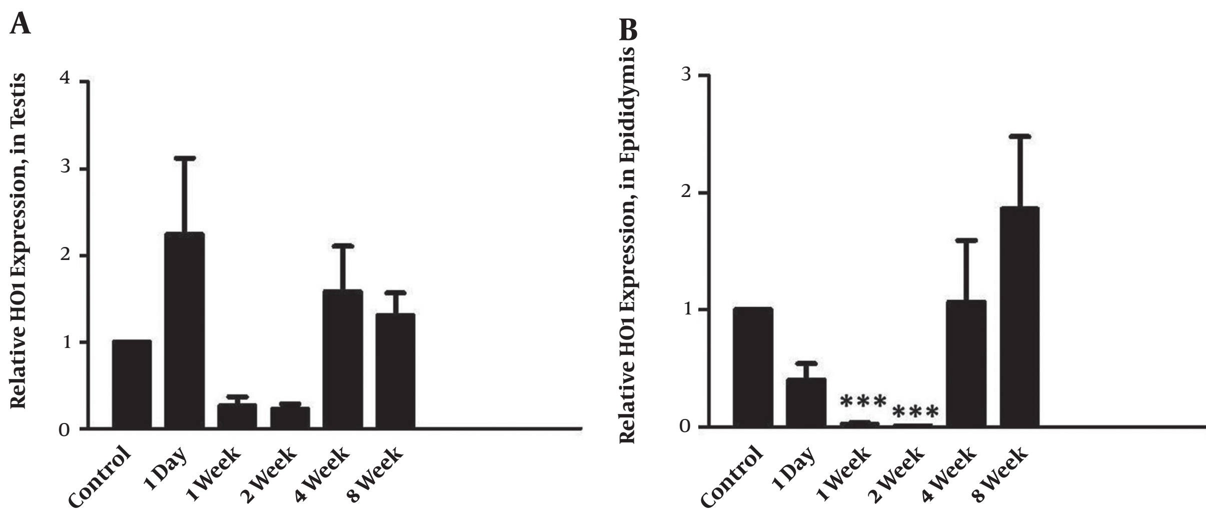

Figure 3. RT-PCR analyzed patterns of HO1 expression in the testis tissue (A) and epididymis (B) of cryptorchid mice at different intervals. *** $P \leq 0.001$

(42°C daily for two hours) could accumulate Nrf2 in the Leydig cells and spermatids only two days after heat exposure (24), and just one day after scrotal heating (42°C daily for 25 minutes), Nrf2 mRNA level up-regulated in the testis tissue (7). On the other hand, cryptorchidism can reduce the number of spermatocytes and spermatids (25). Cryptorchidism can also decrease epithelium height and muscular wall of epididymis (26). This alteration in the number of spermatids and Leydig cells elucidates that the expression of Nrf2 has no changes in testis tissue.

The current study investigated the expression of HO1 and NQO1, two target genes for Nrf2 binding with ARE at their promoter (27, 28). The activity of Nrf2 and the expression of antioxidant genes such as NQO1 and HO1 play protective roles against testicular damage and improve semen quality parameters such as number, motility, and morphology (29, 30). The current study indicated that the expression level of Nrf2, HO1, and NQO1 decreased in epididymis of cryptorchid mice over time. There are limited studies on alteration in Nrf2/Keap1 signaling pathway in gonadal disease; therefore, the current study results need more supporting data to clarify the role of this pathway in male fertility.

In conclusion, the current study data suggested that

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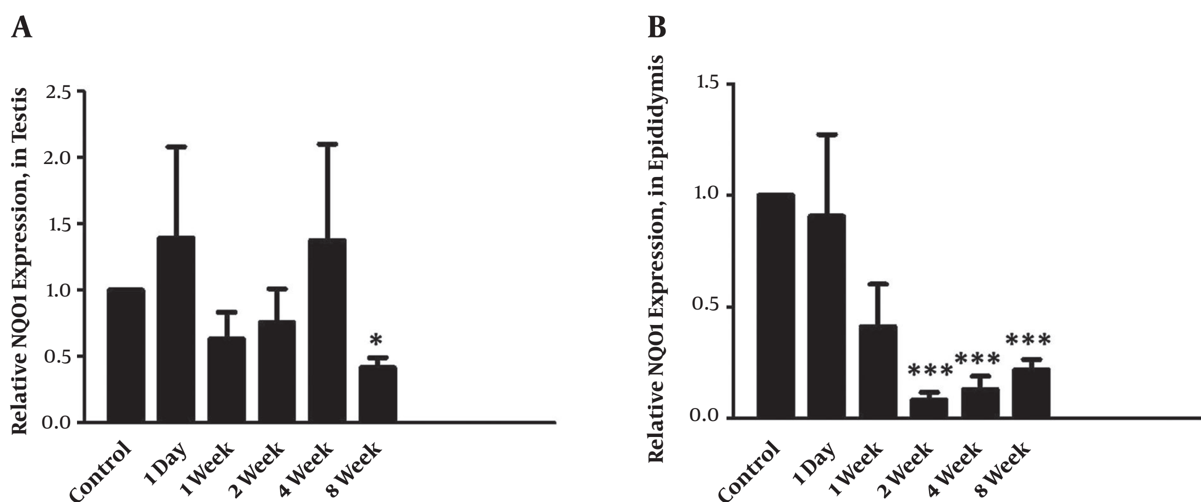

Figure 4. The expression level of NQO1 gene in the testis tissue (A) and epididymis (B) of cryptorchid mice determined by RT-PCR is presented in different groups. * $P \leq 0.05$, *** $P \leq 0.001$

the expression of Nrf2 signaling decreased during cryptorchidism, especially in the epididymis. However, Western blot analysis or immunohistochemistry experiments were not performed. Based on the current study results, it can be hypothesized that using the Nrf2 inducer in cryptorchidism could improve the expression of Nrf2 system in testis and epididymis, and decrease the complications of cryptorchidism.

Acknowledgments

The current study emanated from a proposal approved and supported by the Arak University of Medical Sciences, Arak, Iran.

Footnotes

Authors' Contribution: Hamideh Fallah Asl: performing experiments; Farideh Jalali Mashayekhi: analysis of data and writing of the manuscript; Mohammad Bayat: designing and conducting the study; Danial Habibi: analysis of data; Adib Zendedel: designing the study; Maryam Baazm: designing and conducting the study and writing of the manuscript.

Conflict of Interests: The authors declared no conflict of interest.

Ethical Approval: Research and animal care protocols were approved by the Ethics Committee of Arak University of Medical Sciences (1395.317).

Funding/Support: The present work was financially supported by the Research Deputy of Arak University of Medical Sciences and Health Services Agent under grant No: 2683.

References

1. Kaspar JW, Niture SK, Jaiswal AK. Nrf2:INrf2 (Keap1) signaling in oxidative stress. *Free Radic Biol Med*. 2009;**47**(9):1304–9. doi: 10.1016/j.freeradbiomed.2009.07.035. [PubMed: 19666107]. [PubMed Central: PMC2763938].
2. Li Y, Cao Y, Wang F, Pu S, Zhang Y, Li C. Tert-butylhydroquinone attenuates scrotal heat-induced damage by regulating Nrf2-antioxidant system in the mouse testis. *Gen Comp Endocrinol*. 2014;**208**:12–20. doi: 10.1016/j.ygcen.2014.09.007. [PubMed: 25260249].
3. Durairajanayagam D, Agarwal A, Ong C. Causes, effects and molecular mechanisms of testicular heat stress. *Reprod Biomed Online*. 2015;**30**(1):14–27. doi: 10.1016/j.rbmo.2014.09.018. [PubMed: 25456164].
4. Giudice A, Arra C, Turco MC. Review of molecular mechanisms involved in the activation of the Nrf2-ARE signaling pathway by chemopreventive agents. *Methods Mol Biol*. 2010;**647**:37–74. doi: 10.1007/978-1-60761-738-9_3. [PubMed: 20694660].
5. Li S, Song Z, Liu T, Liang J, Yuan J, Xu Z, et al. Polysaccharide from Ostrea rivularis attenuates reproductive oxidative stress damage via activating Keap1-Nrf2/ARE pathway. *Carbohydr Polym*. 2018;**186**:321–31. doi: 10.1016/j.carbpol.2018.01.075. [PubMed: 29455993].
6. Fawzy F, Hussein A, Eid MM, El Kashash AM, Salem HK. Cryptorchidism and Fertility. *Clin Med Insights Reprod Health*. 2015;**9**:39–43. doi: 10.4137/CMRH.S25056. [PubMed: 26740750]. [PubMed Central: PMC4689328].
7. Li Y, Cao Y, Wang F, Li C. Scrotal heat induced the Nrf2-driven antioxidant response during oxidative stress and apoptosis in the mouse testis. *Acta Histochem*. 2014;**116**(5):883–90. doi: 10.1016/j.acthis.2014.02.008. [PubMed: 24698288].
8. Wajda A, Lapczuk J, Grabowska M, Slojewski M, Laszczynska M, Urasinska E, et al. Nuclear factor E2-related factor-2 (Nrf2) expression and regulation in male reproductive tract. *Pharmacol Rep*. 2016;**68**(1):101–8. doi: 10.1016/j.pharep.2015.07.005. [PubMed: 26721360].

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9. Nakamura BN, Lawson G, Chan JY, Banuelos J, Cortes MM, Hoang YD, et al. Knockout of the transcription factor NRF2 disrupts spermatogenesis in an age-dependent manner. *Free Radic Biol Med*. 2010;**49**(9):1368–79. doi: 10.1016/j.freeradbiomed.2010.07.019. [PubMed: 20692336]. [PubMed Central: PMC2948056].
10. Chen K, Mai Z, Zhou Y, Gao X, Yu B. Low NRF2 mRNA expression in spermatozoa from men with low sperm motility. *Tohoku J Exp Med*. 2012;**228**(3):259–66. [PubMed: 23089668].
11. Sagaradze GD, Basalova NA, Kirpatovsky VI, Ohobotov DA, Grigorieva OA, Balabanyan VY, et al. Application of rat cryptorchidism model for the evaluation of mesenchymal stromal cell secretome regenerative potential. *Biomed Pharmacother*. 2019;**109**:1428–36. doi: 10.1016/j.biopha.2018.10.174. [PubMed: 30551394].
12. Ahangarpour A, Oroojan AA, Heidari H, Ghaedi E, Taherkhani R. Effects of hydro-alcoholic extract from arctium lappa L. (Burdock) root on gonadotropins, testosterone, and sperm count and viability in male mice with nicotinamide/ streptozotocin-induced type 2 diabetes. *Malays J Med Sci*. 2015;**22**(2):25–32. [PubMed: 26023292]. [PubMed Central: PMC4438089].
13. Guzick DS, Overstreet JW, Factor-Litvak P, Brazil CK, Nakajima ST, Coutifaris C, et al. Sperm morphology, motility, and concentration in fertile and infertile men. *N Engl J Med*. 2001;**345**(19):1388–93. doi: 10.1056/NEJMoa003005. [PubMed: 11794171].
14. World Health Organization. *WHO laboratory manual for the examination and processing of human semen*. 2010.
15. Absalan F, Movahedin M, Mowla SJ. Evaluation of apoptotic genes expression and its protein after treatment of cryptorchid mice. *Iran Biomed J*. 2012;**16**(2):77–83. [PubMed: 22801280]. [PubMed Central: PMC3600956].
16. Moretti E, Di Cairano G, Capitani S, Scapigliati G, Baccetti B, Collodel G. Cryptorchidism and semen quality: A TEM and molecular study. *J Androl*. 2007;**28**(1):194–9. doi: 10.2164/jandrol.106.000828. [PubMed: 16988323].
17. Oyewopo AO, Lawal IA, Olawepo A, Enaibe BU, Ajao MS, Oyewopo CI, et al. Cryptorchidism and hyperthermia induced testicular injury in adult male rats; intervention of alpha tocopherol. *Nigerian Biomed Sci J*. 2018:3rd.
18. Ivell R, Hartung S. The molecular basis of cryptorchidism. *Mol Hum Reprod*. 2003;**9**(4):175–81. doi: 10.1093/molehr/gag025. [PubMed: 12651898].
19. Coughlin MT. *Cryptorchidism and male fertility: A study of the determinants of infertility among formerly cryptorchid and control men [dissertation]*. University of Pittsburgh; 2005.
20. Ren L, Medan MS, Ozu M, Li C, Watanabe G, Taya K. Effects of experimental cryptorchidism on sperm motility and testicular endocrinology in adult male rats. *J Reprod Dev*. 2006;**52**(2):219–28. doi: 10.1262/jrd.17073. [PubMed: 16415524].
21. Cheng JM, Tang JX, Li J, Wang YQ, Wang XX, Zhang Y, et al. Role of WNT signaling in epididymal sperm maturation. *J Assist Reprod Genet*. 2018;**35**(2):229–36. doi: 10.1007/s10815-017-1066-4. [PubMed: 29152689]. [PubMed Central: PMC5845038].
22. Montiel EE, Huidobro CC, Castellon EA. Glutathione-related enzymes in cell cultures from different regions of human epididymis. *Arch Androl*. 2003;**49**(2):95–105. [PubMed: 12623745].
23. Kobayashi A, Kang MI, Okawa H, Ohtsuji M, Zenke Y, Chiba T, et al. Oxidative stress sensor Keap1 functions as an adaptor for Cul3-based E3 ligase to regulate proteasomal degradation of Nrf2. *Mol Cell Biol*. 2004;**24**(16):7130–9. doi: 10.1128/MCB.24.16.7130-7139.2004. [PubMed: 15282312]. [PubMed Central: PMC479737].
24. Li Y, Huang Y, Piao Y, Nagaoka K, Watanabe G, Taya K, et al. Protective effects of nuclear factor erythroid 2-related factor 2 on whole body heat stress-induced oxidative damage in the mouse testis. *Reprod Biol Endocrinol*. 2013;**11**:23. doi: 10.1186/1477-7827-11-23. [PubMed: 23514035]. [PubMed Central: PMC3610102].
25. Absalan F, Movahedin M, Mowla SJ. Assessment of testis histological changes and sperm parameters in experimentally-induced unilateral and bilateral cryptorchid mouse model. *Int J Reprod Biomed*. 2008;**6**(3):143–8.
26. De Miguel MP, Marino JM, Gonzalez-Peramato P, Nistal M, Regadera J. Epididymal growth and differentiation are altered in human cryptorchidism. *J Androl*. 2001;**22**(2):212–25. [PubMed: 11229795].
27. Li L, Dong H, Song E, Xu X, Liu L, Song Y. Nrf2/ARE pathway activation, HO-1 and NQO1 induction by polychlorinated biphenyl quinone is associated with reactive oxygen species and PI3K/AKT signaling. *Chem Biol Interact*. 2014;**209**:56–67. doi: 10.1016/j.cbi.2013.12.005. [PubMed: 24361488].
28. Hasanvand D, Amiri I, Soleimani Asl S, Saidijam M, Shabab N, Artimani T. Effects of CeO2 nanoparticles on the HO-1, NQO1, and GCLC expression in the testes of diabetic rats. *Can J Physiol Pharmacol*. 2018;**96**(9):963–9. doi: 10.1139/cjpp-2017-0784. [PubMed: 29894645].
29. Li SG, Ding YS, Niu Q, Xu SZ, Pang LJ, Ma RL, et al. Grape seed proanthocyanidin extract alleviates arsenic-induced oxidative reproductive toxicity in male mice. *Biomed Environ Sci*. 2015;**28**(4):272–80. doi: 10.3967/bes2015.038. [PubMed: 25966753].
30. Maremanda KP, Khan S, Jena G. Zinc protects cyclophosphamide-induced testicular damage in rat: Involvement of metallothionein, tesmin and Nrf2. *Biochem Biophys Res Commun*. 2014;**445**(3):591–6. doi: 10.1016/j.bbrc.2014.02.055. [PubMed: 24565835].

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