

Evaluation of Myc Gene Expression as a Preventive Marker for Increasing the Implantation Success in the Infertile Women

Abstract

Background: There are numerous couples worldwide currently suffering from infertility. Several factors, including genetic abnormalities are involved in infertility. In this study, we investigated the expression of myc gene in uterine tissue of infertile women. The protein encoded by this gene is one of the important transcription factors involved in the expression of many genes in the embryonic growth, and development pathways. **Methods:** There are about 45 samples of uterine tissue from women with primary and secondary infertility were involved in this study. After extracting RNA and synthesizing cDNA, using specific primers for the myc gene and the beta-actin gene (as an internal control), gene expression was evaluated by Real-time RT-PCR method. **Results:** The results of myc gene expression analysis showed no significant pattern between the affected and healthy women, however decreasing of its expression should not be rejected. **Conclusions:** This study is the first report about myc gene expression and its relation with the primary and secondary infertility. Myc gene expression study at different times of sexual period of infertile woman is suggested. Also, we proposed here, as a preventive strategy, improvement of the expression level of myc gene by some methods, such as hormone therapy, can increase the implantation success in the infertile women.

Keywords: Gene expression analysis, myc gene, women infertility

Background

Infertility is defined as the failure to conceive after one year of regular intercourse in women <35 years not using contraception, and after six months in women >35 years.^[1] Epidemiological data suggest that about 10% to 15% of all couples will experience difficulties to conceive (primary infertility) or to conceive the number of children they wanted (secondary infertility). Based on a survey performed in developed countries, the World Health Organization (WHO) estimates that female infertility accounts for 37% of causes in infertile couples, male infertility for 8% and both – male and female infertility – for 35%. About five percent of couples have unexplained infertility and 15% became pregnant during the study.^[2] Involvement of genetic factors in the human and the other mammals infertility is reported by thousands papers. In some of studies, expression of Hundreds genes is correlated with infertility.^[3-5] The protein encoded by myc gene is a multi-functional phosphoprotein

that plays a role in the progression of cell cycle, growth, and apoptotic cell development. Myc gene encoded protein is a nuclear proto-oncogene that is essential for growth and differentiation.^[6,7] Importance of the expression and mutation of this gene during pregnancy and pre implantation period has been reported by some studies. In addition, the secretion of ovarian steroids is associated with the expression of the myc gene.^[8,9] The increased expression of myc gene has been reported in several cancers. Gain of function mutations of this genes have been identified in the cervical, clonal, breast and stomach cancer cells.^[10] These reports emphasis on the importance of study of the expression of myc gene and probable correlation of it with the infertility. This theme is sometimes more interesting to know this gene contributes to regulating 15 percent of human genes. Myc gene act as a gene regulator, through the binding to the enhancer boxes in the DNA level, in addition to activating of histone acetyltransferase in the chromatin level.^[11] This role can be critical for primary process of pregnancy such as

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implantation. The aim of this study is to determine the expression of myc gene in uterine tissue of women with asymptomatic infertility.

Methods

Samples collection, RNA extraction

A total of 45 samples of myometrium tissue were dissected from uterine tissue of women (mean age 28 between 25-39 years) registered in Hajar Hospital in Shahrekord, affected by infertility (N = 30) and normal (N = 15) with their consent. The myometrial samples transferred to the laboratory in freeze condition and stored at -70°C. Total RNA was extracted by using TRIZOL reagent (Thermo-Fisher Scientific Co. USA) according to the manufacturer's instructions. Extracted RNA was treated with DNase I in order to removing of genomic DNA contamination.

cDNA synthesis and real time –RT-PCR

First-strand cDNA was synthesized using oligo dT primers and random hexamer as recommended by Takara kit first-strand cDNA synthesis manual (Takara Co. Japan). In order to optimization of realtime-RT-PCR, standard RT-PCR was carried by using of forward and reverse primers [Table 1] for myc as target gene and β -actin gene as internal control and reference gene. The reactions was optimized separately under the following thermal conditions: 95°C for 5 minutes (initial denaturation), followed for 35 cycles 94°C for 30 seconds, 60°C for 30 seconds and 72°C for 30 seconds. RT-PCR products were analyzed on 1% agarose gel electrophoresis using 1X TBE buffer in a constant voltage of 85 V for 40-50 minutes. Gel staining was performed by using greenviewer (Aron gene Co. Iran) and visualized by UV-Doc (Nogen Co. Iran).

Real time –RT-PCR was performed by using 5 μ l SYBER Green reagent (Takara, Japan), 50 ngr synthesized cDNA, forward and reverse primers up to final concentration equal with 0.4 μ M in total volume of 10 μ l. Thermal condition was performed as described for standard RT-PCR in Corbett Rotorgene 6000 apparatus (Bosch institute, Australia).

Statistical analyses

In the present study, to compare the number of copies of target gene and reference gene, a comparative method was used according to the following formula:

$[\Delta\Delta Ct = (Ct \text{ target gene} - Ct \text{ reference gene}) \text{ Unknown sample} - (Ct \text{ target gene} - Ct \text{ reference gene}) \text{ Normal control}]$.

Data analysis was performed using SPSS version 23. The *P* value was calculated by T-test to determine the statistical difference between the two groups. Also, REST software was used to confirm the results obtained at *P* value.

Results

Total RNA was extracted from uterine tissue and standard RT-PCR was performed. RT-PCR products were analyzed in 1% agarose gel electrophoresis (related data are not shown here).

Real time RT-PCR

After performing PCR and Verify of the cDNAs, in order to examine the gene expression, the cDNAs were applied for Real-Time reaction. Melting curve and amplification plot of the mycas target gene and the beta-actin as reference gene are shown in Figure 1.

Relative quantification data analysis

Figures 2,3 and Table 2 show the descriptive results of Real time RT-PCR reactions between patients and control groups.

Statistical analysis

In statistical analysis of the data, first, to determine whether the data follows normal distribution or not Kolmogorov-Smirnov test was used. Given that the Kolmogorov-Smirnov test showed that the mean of the expression data follows a normal distribution, therefore, independent T-test was used to obtain a meaningful difference between two groups. In this test, the significance level of *P* = 0.13 was obtained that showed the mean of gene expression between two groups of patients and normal people did not have a significant difference. Also, in order to the more confidence, the *P* value was calculated in the REST software and this results were similar to the results the results came from T-test. Our results based on comparative $\Delta\Delta Ct$ and target gene ratio to reference gene) $2^{-\Delta\Delta Ct}$, showed there was no significant difference between the two groups (*P* value >0/001). However, calculation of fold change (FC) in mRNA expression level ($2^{-\Delta\Delta Ct}$, showed a decreasing by 1.69 times in myc gene expression in the patients compared to the normal group [Table 2 and Figure 3].

Table 1: Primer sequences and their properties applied for analysis of Myc and β -Actin genes

Primer name	Sequence	Target gene	Amplicon length	Accession Number in NCBI
FTMY1	5- GAGGAGACACCGCCACCAC -3	C-Myc	125bp	NM_002467
RTMY1	5- GAAGGTGATCCAGACTCTGAC-3			
ActF	5'-GGCGGCACCACCATGTACCC-3'	β -Actin	120bp	NM_001101.3
ActR	5'- CCACACGGAGTACTTGCGC -3'			

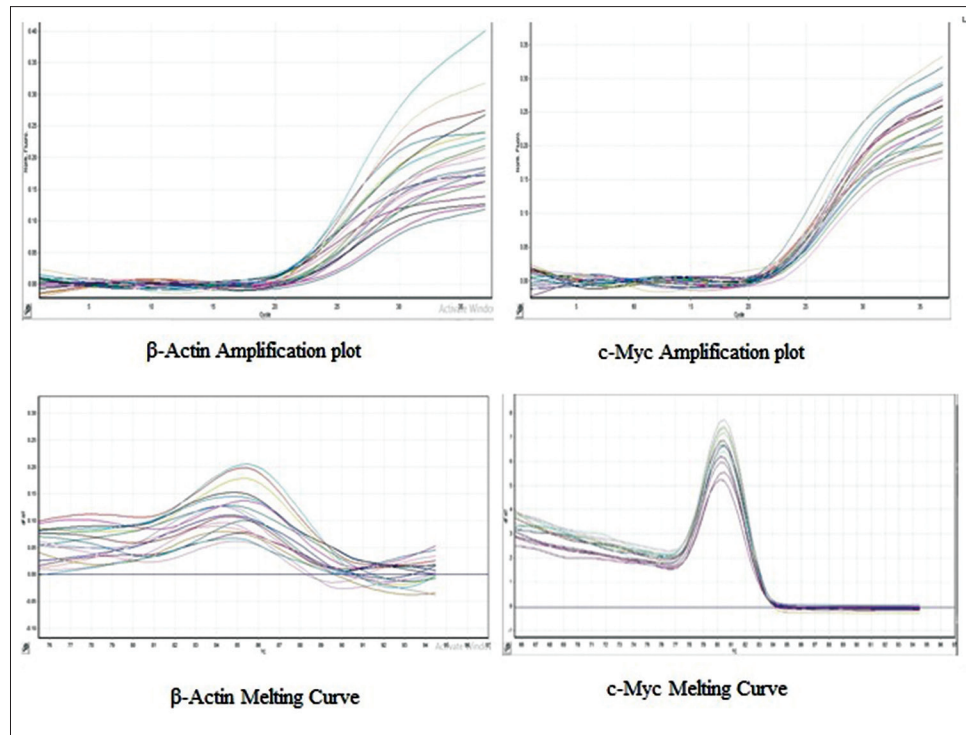


Figure 1: Melting and amplification diagrams related to the myc and β -actin genes

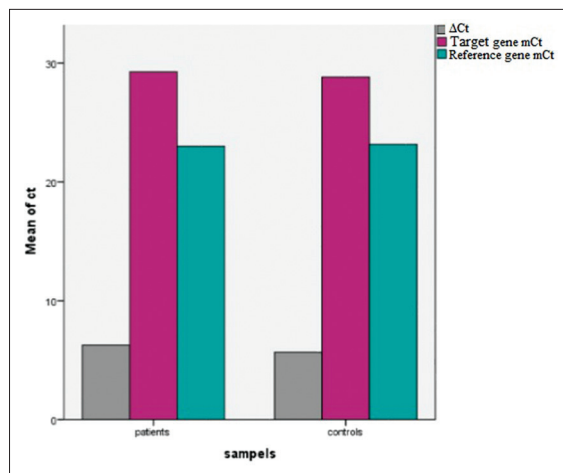


Figure 2: Descriptive comparison of mCt ratios in patients and normal individuals

Discussion

Infertility is a common clinical problem in humans that has a similar outbreak of life-threatening conditions like diabetes or hypertension.^[12,13] Molecular study of female infertility using Real time RT-PCR demonstrates the involvement of many key genes involved in endometrial changes in early stages of pregnancy. After each menstrual period, endometrial wall is only ready for embryos implantation for 3-5 days during the female sexual period that this time is called the "implantation window".^[14,15] Investigating the expression of genes and factors involved in uterine preparation for embryo acceptance, can clarify the cause of some infertility

Table 2: The comparative expression analysis based on obtained $\Delta\Delta Ct$ between patient and normal groups

Samples	<i>n</i>	Mean \pm SD	Student <i>t</i> -test
Control	15	5.220.77	<i>P</i> >0/001
Patients	30	6.00	

*There was no meaningful evidence variance between patient and control group. However we can propose difference in expression level myc gene between two groups

problems. Our study that is presented in this article focuses on one of these important molecular pathways.

In humans, implantation involves complex interactions between the embryo and the endometrium of the mother, all of which must be done in a desired time frame. The results of endometrial gene expression in the women with asymptomatic infertility showed a significant relationship between the results and the period time in which the endometrial biopsy specimens were taken.^[16] This significance is well documented in the study of SRK1 gene expression. Expression of this gene in endometrial biopsy specimens collected from the patients in 5th to 10th days of the LH phase, did not show any significant difference against the control group.^[17] These results can support our study. In our study, the expression of the myc gene, which is very important in controlling the expression of genes involved in development, no significant difference was seen between cases and controls. Although, the similarity of the samples based on their conditions is well thought out in our study and according to the relevant specialist, all samples were collected from the women who were

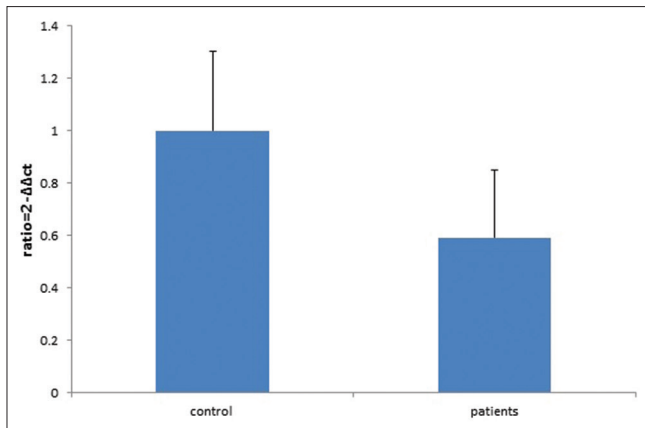


Figure 3: The ratio of the number of copies of the target gene to the reference gene in the healthy subjects and the patients, which did not show significant difference between the two groups ($P > 0.001$). However, the difference is evident

in their final days of menstrual cycle. Accordingly, one of the possible reasons for absence of the meaningful difference in expression of this gene with the control group is the selected period. Also, in our study, the sampling was carefully monitored by an obstetrician from the uterus fundus. Therefore, we can claim that the focal expression of myc gene is also considered in our study. In other words, it might be better to use sample tissue from the other area of endometrium or even placenta in myc gene expression analysis. The focal and time-dependent nature of the expression of genes in other studies has also been emphasized and supports our claim.^[18] Result of a study by Altmae *et al.* on the gene expression of uterine tissue in women with asymptomatic infertility, showed increasing the expression of 145 genes and reducing the expression of 115 genes compared with the control group.^[5] The myc gene examined in our study has not been considered in other studies and our work is the first report the expression analysis of this gene in the uterine tissue. However, the presence of the myc gene in the gene pathways studied by Altmae *et al.* and the similarity of the subjected patients, confirms the importance of the changes in the expression of the myc gene, which should be considered. This proposal is supported by result of Zhao *et al.* works that were proven the role of ERK/JNK/MAPK pathway in deactivation of myc gene.^[19] We have studied myc gene in our study, which has received less attention in previous studies and the expression of this gene in uterine tissue has not been considered with primary and secondary infertility. It seems that the loss of function mutation of this gene, resulting in a reduction or lack of expression, is inconsistent with the survival of the fetus, in other words, the absence of reports about these type of mutations is not meaning that they does not occur. On the other hand, gene expression studies face several constraints and such studies can be complex and unpredictable in many aspects. For example, tissue specific pattern for the expression of several genes in the uterine

wall have been reported.^[20-22] and it may be the expression of the myc gene in our study follows such a pattern. In this study, selection of the patients based on our criteria took a lot of time, so, the results have less segregation. In our study, women were somewhat characterized for infertility reasons and cases involving karyotype disorders or the history of uterine and ovarian diseases were dropped. We suggest that further studies should be carried out on more sample sizes. In addition, sampling from the different regions of the uterus and even oviduct should be done. We propose monitoring the hormonal regimen of women with infertility as supplementary information. Studies have shown that steroid hormones such as estrogen and progesterone increase the expression of the myc gene.^[23,24] Based on this, we suggest that tissue harvests take place in minimal hormonal periods. The $\Delta\Delta C_t$ method used in this study has been supported in several studies in terms of the advantage compared with other methods.^[25,26] Given that the Kolmogorov-Smirnov test showed that the mean of data follows a normal distribution, independent T-test was used to obtain a meaningful difference between two groups. In the statistical test, a significant level of $P = 0/13$ was founded that means there was no significant difference between mean of expression of genes between normal and patient groups, however, the reduction of myc gene expression with a 1.69 coefficient found in our study, should be considered. Several studies have reported that progesterone and estrogen hormones can lead to the overexpression of myc gene.^[27,28] Regard to this results, we proposed here, as a preventive strategy, improvement of the expression level of myc gene by some methods, such as progesterone and estrogen therapy, can lead to a more effective expression level of the genes that have important role in successful implantation.

Conclusions

This study is the first report on the correlation between the expression of myc gene and primary and secondary infertility in the women. Although the expression of this gene in the present study did not show a meaningful relationship between the patient and the control group but due to the importance of the myc gene as a transcription factor, it cannot be argued that this gene has not any role as a Prognostic factor in the women infertility. Sample size and the variance between the type of patients and time of sample collection should be considered.

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Conflicts of interest

There are no conflicts of interest.

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References

- Anwar S, Anwar A. Infertility: A review on causes, treatment and management. *Womens Health Gynecol* 2016;2:40-5.
- WHO: The influence of varicocele on parameters of fertility in a large group of men presenting to infertility clinics. *World Health Organization. Fertil Steril* 1992;57:1289-93.
- Achermann JC, Ozisik G, Meeks JJ, Jameson JL. Genetic causes of human reproductive disease. *J Clin Endocrinol Metab* 2002;87:2447-54.
- Maston GA, Evans SK, Green MR. Transcriptional regulatory elements in the human genome. *Annu Rev Genom Human Genet* 2006;7:29-59.
- Matzuk MM, Lamb DJ. Genetic dissection of mammalian fertility pathways. *Nat Cell Biol* 2002;4:41-9.
- Pelengaris S, Khan M. The many faces of C-Myc. *Arch Biochem Biophys* 2003;416:129-36.
- Huang CY, Bredemeyer AL, Walker LM, Bassing CH, Sleckman BP. Dynamic regulation of Myc proto-oncogene expression during lymphocyte development revealed by a GFP-Mycknock-in mouse. *Eur J Immunol* 2008;38:342-9.
- Huet-Hudson YM, Andrews GK, Dey SK. Cell type-specific localization of Myc protein in the mouse uterus: Modulation by steroid hormones and analysis of the periimplantation period. *Endocrinology* 1989;125:1683-90.
- Davis AC, Wims M, Spotts GD, Hann SR, Bradley A. A null Myc mutation causes lethality before 10.5 days of gestation in homozygotes and reduced fertility in heterozygous female mice. *Genes Dev* 1993;7:671-82.
- Finver SN, Nishikura K, Finger LR, Haluska FG, Finan J, Nowell PC, *et al.* Sequence analysis of the MYC oncogene involved in the t (8;14) (q24;q11) chromosome translocation in a human leukemia T-cell line indicates that putative regulatory regions are not altered. *Proc Natl Acad Sci* 1988;85:3052-6.
- Cotterman R, Jin VX, Krig SR, Lemen JM, Wey A, Farnham PJ, *et al.* N-Myc regulates a widespread euchromatic program in the human genome partially independent of its role as a classical transcription factor. *Cancer Res* 2008;68:9654-62.
- Gurunath S, Pandian Z. Defining infertility—A systematic review of prevalence studies. *Hum Reprod Update* 2011;17:575-88.
- Hochschild FZ, Adamson GD, Dyer S, Racowsky C, Mouzon J, Soko R, *et al.* The international glossary on infertility and fertility care, 2017. *Fertil Steril* 2017;108:393-406.
- Reese J, Das SK, Paria BC, Lim H, Song H, Matsumoto H, *et al.* Global gene expression analysis to identify molecular markers of uterine receptivity and embryo implantation. *J Biol Chem* 2001;276:44137-45.
- Altmäe S, Reimand J, Hovatta O, Zhang P, Kere J, Laisk T, *et al.* Interactome of human embryo implantation: Identification of gene expression pathways, regulation, and integrated regulatory networks. *Mol Endocrinol* 2012;26:203-17.
- Altmäe S, Martínez-Conejero JA, Salumets A, Simón C, Horcajadas JA, Stavreus-Evers A. Endometrial gene expression analysis at the time of embryo implantation in women with unexplained infertility. *Mol Hum Reprod* 2010;16:178-87.
- Feroze-Zaidi F, Fusi L, Takano M, Higham J, Salker MS, Goto T, *et al.* Role and regulation of the serum- and glucocorticoid-regulated kinase 1 in fertile and infertile human endometrium. *Endocrinology* 2007;148:5020-9.
- Creus M, Ordi J, Fábregues F, Casamitjana R, Ferrer B, Coll E, *et al.* $\alpha\beta 3$ integrin expression and pinopod formation in normal and out-of-phase endometria of fertile and infertile women. *Hum Reprod* 2002;17:2279-86.
- Zhao Q, Assimopoulou AN, Klauk SM, Damianakos H, Chinou I, Kretschmer N, *et al.* Inhibition of myc with involvement of ERK/JNK/MAPK and AKT pathways as a novel mechanism for shikonin and its derivatives in killing leukemia cells. *Oncotarget* 2015;17:6:3893451.
- Warrington JA, Nair A, Mahadavappa M, Tsyganskaya M. Comparison of human adult and fetal expression and identification of 535 housekeeping/maintenance genes. *Physiol Genomics* 2000;2:143-7.
- Nishimura M, Naito S. Tissue-specific mRNA expression profiles of human toll-like receptors and related genes. *Biol Pharm Bull* 2005;28:886-92.
- Fagerberg L, Hallström BM, Oksvold P, Kampf C, Djureinovic D, Odeberg J, *et al.* Analysis of the human tissue-specific expression by genome-wide integration of transcriptomics and antibody-based proteomics. *Mol Cell Proteomics* 2014;13:397-406.
- Okubo T, Urabe M, Tsuchiya H, Iwasa K, Yokota K, Kikuchi N, *et al.* Effect of estrogen and progesterone on gene expression of growth regulatory molecules and proto-oncogene in vascular smooth muscle cells. *Endocr J* 2000;47:205-14.
- Wang C, Mayer JA, Mazumdar A, Fertuck K, Kim H, Brown M, *et al.* Estrogen induces myc gene expression via an upstream enhancer activated by the estrogen receptor and the AP-1 transcription factor. *Mol Endocrinol* 2011;25:1527-38.
- Demes M, Bartsch H, Scheil-Bertram S, Mücke R, Fisseler-Eckhoff A. Real-time PCR data processing shown by the analysis of colorectal specific candidate genes, ERCC1, RRM1 and TS in relation to $\beta 2M$ as endogenous control. *Appl Sci* 2012;2:139-59.
- Rao X, Huang X, Zhou Z, Lin X. An improvement of the $2^{-\Delta\Delta CT}$ method for quantitative real-time polymerase chain reaction data analysis. *Biostat Bioinforma Biomath* 2013;3:71-85.
- Wang C, Mayer JA, Mazumdar A, Fertuck K, Kim H, Brown M, *et al.* Estrogen induces c-myc gene expression via an upstream enhancer activated by the estrogen receptor and the AP-1 transcription factor. *Mol Endocrinol* 2011;25:1527-38.
- Okubo T, Urabe M, Tsuchiya H, Iwasa K, Yokota K, Kikuchi N, *et al.* Effect of estrogen and progesterone on gene expression of growth regulatory molecules and proto-oncogene in vascular smooth muscle cells. *Endocr J* 2000;47:205-14.