Inverse Regulation of Mir Let-7g and HMGA2 Gene in HPV-Infected and Not Infected Cervical Cancer Patients

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Authors' contributions

This work was carried out in collaboration between all authors. Author FB wrote the protocol and designed the study. Author KO performed the statistical analysis, wrote the first draft of the manuscript and managed the analyses of the study. Author AMM managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Introduction: Cervical cancer is the fourth most common neoplasm among women worldwide, with the majority of cases occurring in developing countries. High-risk human papilloma viruses (HPVs) types are accounted for more than 90% of cervical carcinomas. Despite standard treatment for cervical cancer, no specific molecular markers can predict the clinical response of patients diagnosed yet. Decreased in the miRNAs Let-7 family expression is reported to be associated with cervical cancer and Re-expression of that inhibits the proliferation and migration of cells via $HMGA_2$ in carcinoma. The aim of the current study was to determine the correlation of the miR Let-7g and $HMGA_2$ gene expression in HPV-infected cervical cancer patients and compare it with not infected patients and control groups among participating refereed to MirzaKochak Khan Hospital, Tehran, Iran, 2015.
Material and Methods: Total of 60 samples including 20 HPV-infected cervical cancer, 20 patients with no HPV infection and 20 healthy individuals as control were collected. Cervical cancer was

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determined by Pap smear and tissue sampling tests. The HPV 16 and 18 types were investigated by Real Time High-Risk HPV assay. The RNA was extracted, quality and quantity of the RNA were determined and the expression of the Let-7g, $HMGA_2$ and GAPDH genes were determined using Real-time quantitative PCR (qRT-PCR) and $\Delta\Delta CT$ computational techniques. Data were analyzed using Graphpad Prism 6 software.

Result: According to the results, miR Let-7g expression decreased in HPV infected and not infected cervical cancer patients compare to control group (P=0.0002, P=0.0132 respectively). We found a significant decrease in miR Let-7g expression in HPV infected compare to not infected patients (P=0.0001). We observed an increased $HMGA_2$ gene expression in infected and not infected cervical cancer patients compared to healthy individuals (P=0.0001, P=0.0005 respectively). Also, a positive association was observed with increased in $HMGA_2$ gene expression in HPV infected patients compare to not infected women (P=0.0001).

Conclusion: these results suggested miR Let-7g along with the $HMGA_2$ gene is a useful indicator for early prognosis of the HPV infected cervical cancer.

1. INTRODUCTION

Cervical cancer progress from the precursor stage, manifested by cervical intraepithelial neoplastic lesions, to invasive tumors [1] and it ranks as the fourth most common neoplasm among women worldwide, with the majority of cases occurring in developing countries, and it is responsible for more than 260,000 yearly deaths [2]. Despite standard treatment as radiotherapy and chemotherapy introduced for treatment of cervical cancer, no specific molecular markers can predict the clinical response of patients diagnosed yet [3]. Among high-risk human papilloma viruses (HPVs) types (16, 18, 31, 33, 34, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 and 70), HPV-16, 18 and 31 are account for more than 90% of cervical carcinomas [1]. HPVs are classified into high- and low-risk types which HPV-16 infection known as high-risk HPV type. Clinical studies suggested the HPV-16 infection is an important risk factor for cancers [4]. Two main viruses encode transforming proteins of the HPV are known as E6 and E7. The E7 protein binds to hypophosphorylated pRb and destabilizes pRb, resulting in disruption of Rb/E2F complexes [1]. These proteins neutralize p53 and Rb tumour suppressor pathways, respectively [1]. Deregulation of the expression of HPV oncogenes E6 and E7 play essential role in cervical carcinogenesis.

The miRNAs are a group of short and non-coding RNAs, situated within the introns or exons of coding genes. MicroRNAs are associated with the cell cycle, apoptosis, proliferation, differentiation and metabolic pathways [5]. These molecules regulate the expression of their mRNA targets. Some microRNAs expression increased which target tumour suppressor protein known as oncomirs and those with a decreased expression which generally target oncogenes, referred to as tumour suppressor microRNAs [6]. MicroRNA Let-7 was primarily found to regulate cell differentiation and proliferation in *Caenorhabditis elegans* and it is a kind of highly conserved miRNAs across species and detected in multiple genomic locations [7]. The Let-7g family includes nine members, Let-7a, Let-7b, Let-7c, Let-7d, Let-7e, Let-7f, Let-7g, Let-7i and miR-98 [8]. Let-7g family plays a key role in regulation, development and carcinogenesis of numerous target genes such as rat sarcoma (RAS), Myc, $CCND_1$, LIN_{28} and HMGA [8]. The miRNAs genes expressed in the cancer-associated genome regions and Let-7 family act as tumour suppressor miRNAs which regulate expression of many oncogenes [9]. Decreased in the Let-7 family expression has been reported but in some cases high expression levels are associated with human cancers (Ali et al. 2010). Decreased Let-7 family function is associated with various cancers [10]. High-mobility group A (HMGA) proteins are polypeptides of ~100 amino acid residues and including 3 subtypes, $HMGA_{1a}$, $HMGA_{1b}$ and $HMGA_2$. These proteins regulate gene expression by altering the structure of chromatin or direct protein-protein interactions with transcription factors [11]. HMGA proteins have interacting domains which are able to modify macromolecular complexes and biological processes [11]. Also, the HMGA proteins participate in the regulation of many genes including interferon-β, NF-κB and interleukins [11]. HMGA is reported to be a target oncogene for the miRNAs Let-7 [10].

Numerous microRNAs with altered expression in cervical cancer have been identified [6]. miRLet-7 has anti-carcinogenic properties in a variety of tissues by the act on the pro-proliferative and/or pro-metastatic targets, such as HMGA$_2$ [12]. Recent studies suggested that Let-7 is crucial in the occurrence and development of the HPV-associated cancers [4]. For instance, HPV-16 promotes cell growth of oesophagal cancer via down-regulation of microRNAs [4]. A correlation reported between the HMGA and miR Let-7 which HMGA is oncogenes critical in tumorigenesis, proliferation and invasion which are targeted by Let-7 [10]. These oncogenes activate or up-regulate the expression of their downstream target proteins, which directly regulate the cell cycle, apoptosis and cell adhesion. Let-7 may thus act as a tumour suppressor through the inhibition of these oncogenic signalling pathways [10]. Over expression of miR Let-7, might block the tumour progression and perhaps promotes the apoptosis by interacting in the target genes [13]. Re-expression of miR Let-7, inhibits the proliferation and migration via HMGA in hepatocellular carcinoma [14]. So, the members of the Let-7 family are associated with numerous cancers and can use as diagnostic, predictive and prognostic biomarkers [15]. Therefore, the aim of the current study was to determine the correlation between miR Let-7 and HMGA$_2$ gene expression in HPV-infected and not infected cervical cancer patients and compares it with healthy individuals.

2. MATERIALS AND METHODS

2.1 Patients and Samples

In this case-control study, 20 HPV-infected cervical cancer women, 20 patients with no infection and 20 normal cases, were collected from Mirza KuchekKhan Hospital, Tehran, Iran in 2015. All participants were aged between 21-46 years. The volunteers had no previous use of anti-pregnancy medications or smoking and immune system disorders at least for past 5 years. The Pap smear and tissue sampling tests were done in all patients to determine the incidence of cervical cancer. Then the Pap smear results compared with their histopathology report to determine invasive carcinoma.

2.2 HPV-16 and HPV-18 Infections

To determine the HPV types 16 and 18 infections, Real Time High-Risk HPV assay was done using Technology-DNA PCR Amplification Kit (HPV 16 & 18 lot: 8045) based on the manufacture instructions. The HPV 16 and 18 genotype test results interpreted in conjunction with results from HPV 16 and 18 testing and cervical cytology [16]. Briefly, After PCR, the samples allocated into flash PCR Gene detector (Technology-DNA) and results reported by Gene v4 software. The patients who were no invasive carcinoma and negative HPV 16 and 18 infections allocated to control (normal) group. The other patients allocated into HPV positive or negative groups.

2.3 RNA Extraction and cDNA Synthesis

The tissue samples deparaffinized using xylene (1000 µl) at 37ºC for 5 min. Then micro tubes centrifuged at 3800 rpm for 5 min and the supernatant removed. Then ethanol (1000 µl) was added and inverted for 5 min, centrifuged at 13000 rpm at 6ºC for 5 min. The ethanol and xylene of the micro tubes entirely removed. The total RNA was extracted, using the RNX plus™ kit according to the manufacturer's recommendations (Cinnagen, Tehran, Iran). Briefly, 100 µl of the tissue sample homogenized with the RNX-PLUS solution (500 µl) and incubated at room temperature for 5 min. Chloroform (200 µl) was added to the solution and centrifuged for 15 min at 12000 rpm. The upper phase was then transferred to another tube and an equal volume of isopropanol was added. The mixture was centrifuged for 15 min at 12000 rpm and the resulting pellet was washed in ethanol (70%) and dissolved in DEPC-treated water. The purity and the integrity of the extracted RNA were evaluated by optical density measurements and visual observation of sample electrophoresis on 2% agarose gel [17] using NanoDrop spectrophotometer with 260 and 280 nm for DNA and RNA, respectively (Jiang et al. 2010). cDNA was synthesized from total RNA using the Taqman miRNA reverse transcription kit. The Random Hexamer used as a primer (1 µl) and the Oligo DT (1 µl) were added vortex and spine. Then 10 µl of RNA added and incubated at 65ºC for 5 min. Then water nucleus free (4.5 µl), MMULV buffer (2 µl) and MMULV (0.5 µl) were added to the final volume of 20 µl.

2.4 Real-time PCR

Real-time quantitative PCR (qRT-PCR) was performed using the Applied Biosystems 7500 Sequence Detection system (Applied Biosystems, USA). The expression of Let-7g,

$HMGA_2$ and GAPDH genes were defined based on the threshold cycle (Ct), and relative expression levels were calculated after normalization with reference to expression of small nuclear RNA [18]. The steps of mRNA RT-PCR were performed as described by Kaka et al. [17]. Briefly, 1ng of total RNA obtained from samples transcribed with oligo (dT) and then RT-PCR was performed. The primers (sequences 5´→3´) used for Let-7g, $HMGA_2$ and GAPDH genes were listed as follows: Let-7g-forward: GTTTGGTGAGGTAGTAGTTTGT (51.11°C), Let-7g-reverse: GTGCAGGGTCCGAGGT (51.06°C); $HMGA_2$-forward: AAAGCAGCTCAAAAGAAAGCA, $HMGA_2$-reverse: TGTTGTGGCCATTTCCTAGGT; GAPDH-forward: ATGGAGAAGGCTGGGGCT; GAPDH-reverse: ATCTTGAGGCTGTTGTCATACTTCTC (Wang and Ruan, 2010). The cycling conditions for Let-7g and GAPDH were as follows: initial denaturation at 95°C for 10 seconds followed by 35 cycles of the 95°C and a final extension of GAPDH (59°C), Let-7g (58°C) at 15 seconds. For amplification of $HMGA_2$, reverse transcription PCR was programmed as follows: 95°C for 2 min, 30 cycles of 94°C for 30 s, 56°C for 30 s, 72°C for 45 s, 72°C for 10 min, hold at 4°C. Each experiment was repeated at least 3 times in order to ensure reproducibility. The size of the digested products was checked on 2% agarose gel electrophoresis. The relative expression levels of Let-7g and $HMGA_2$ were calculated using the comparative $2^{\Delta\Delta Ct}$ methods [19]. The fold changes in these genes were calculated using the ΔΔCt method. All experiments were performed at least in triplicate [19].

2.5 Statistical Analysis

The data of Let-7g, $HMGA_2$ and GAPDH genes expression between groups were subjected to the *t*-Student and Mann–Whitney tests. Statistical analysis was carried out using IBM SPSS Statistics for Windows, Version 22.0 (IBM Crop., Armonk, NY, USA) and GraphPad Prism 6 software. Data are presented as mean ± SEM and P<0.05 was considered statistically significant.

3. RESULTS

The results of the DNA genomic and RNA extraction in agarose gel are presented in Figs. 1 and 2.

To determine the specification of the primers and florescence colour, as well as ensure to specific amplification in PCR product, the melt curve of the Let-7g, $HMGA_2$ and GAPDH genes were determined and presented in Figs. 3-5, respectively.

After the amplification reaction, the Ct of the samples converted to the relative quantification and determined using the ΔΔCt method. The RQ of the obtained results of the HPV-infected and not infected cervical cancer patients were compared with healthy individuals. The result of the miR Let-7g expression is presented in Fig. 6. A significant difference detected in the miR Let-7 g expression among HPV-infected patients compared to the control group (P=0.0002). Also, a positive association was observed between decreased miR Let-7g expression in not infected cervical cancer patients compared with healthy individuals (P=0.0132). Comparison between two infected and not infected HPV groups showed a significant decreased in Let-7g expression among HPV-infected compared to not infected cervical cancer patients (P=0.0001).

Based on the Fig. 7, the $HMGA_2$ gene expression was increased among HPV infected and not infected patients compared to a normal group which they were statistically significant (P=0.0001, P=0.0005 respectively). We found an increased association between the $HMGA_2$ gene expression in HPV infected compared with not infected cervical cancer patients (P=0.0001).
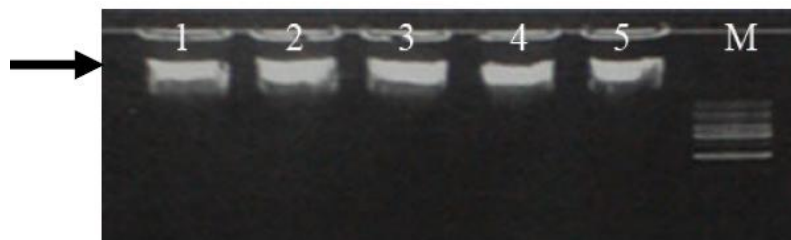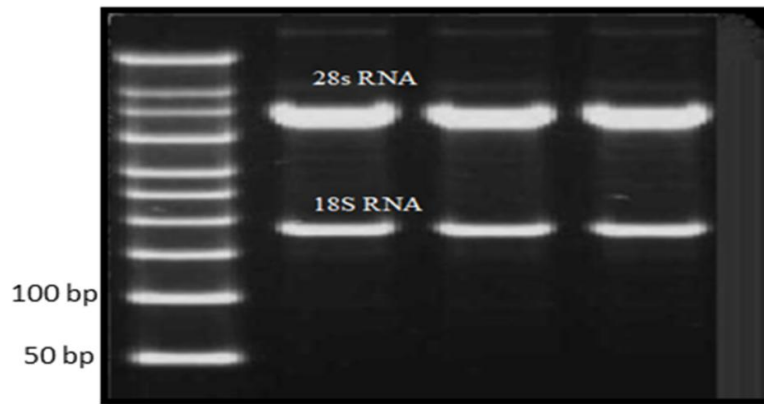

Fig. 1. The DNA genomic extraction in agarose gel
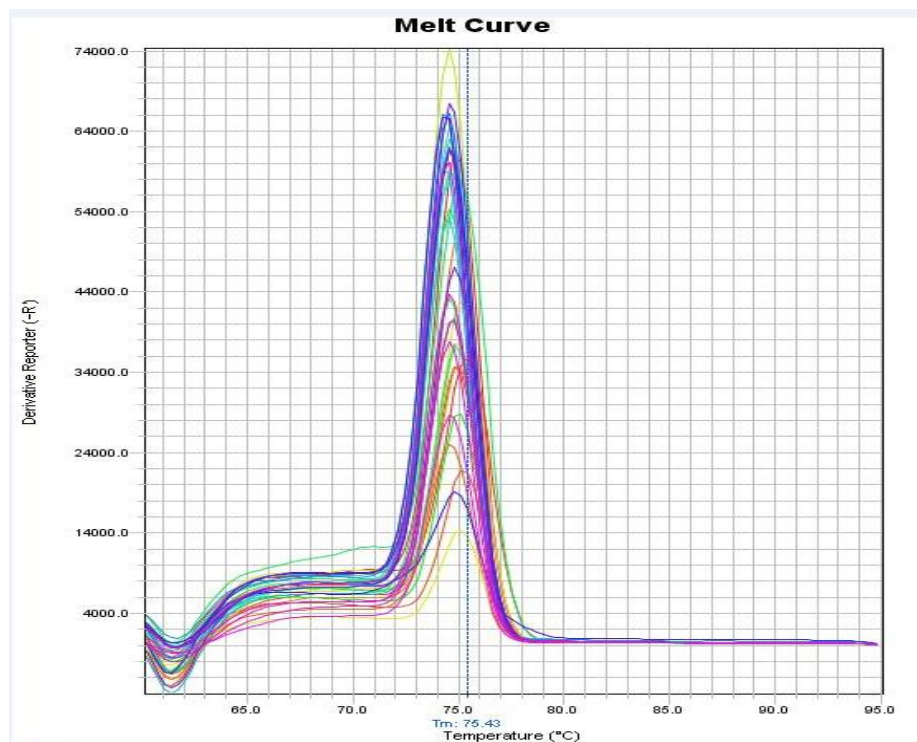
Fig. 2. The extracted RNA in agarose gel

Fig. 3. The melt curve of the Let-7g

On the base of age, there was no any significant association between miR Let-7g expression in cervical cancer patients aged >35 compared to <35 years old (P=0.590). A significant difference was detected in the expression of miR Let-7g in IC (10.78±6.48) compared to C3 (14.77±2.87) stages (P=0.016). Regarding HMGA$_2$ gene, we found no positive association between this gene expression neither with age nor with the stage of disease in the groups among the studied population (P>0.05).

4. DISCUSSION

The link between genital HPV infections and cervical cancer is known for many decades [3]. HPVs are presented in 90% of the cervical cancers and types 16 and 18 are the most common high-risk types associated with cervical cancer [20]. Transcription of the E6 and E7 genes is under control of the core-enhancer that forms HMGA$_2$. Full activation of the core-enhancer integrated into the host genome may

require interchromosomal interaction with a still unknown regulatory element [1]. Despite Pap testing has acceptable results in cervical cancer detection, but approximately 25% death was reported in false-negative HPV patients [20]. So, it is growing interest in HPV DNA molecular testing in women with cervical lesions [21; 22].
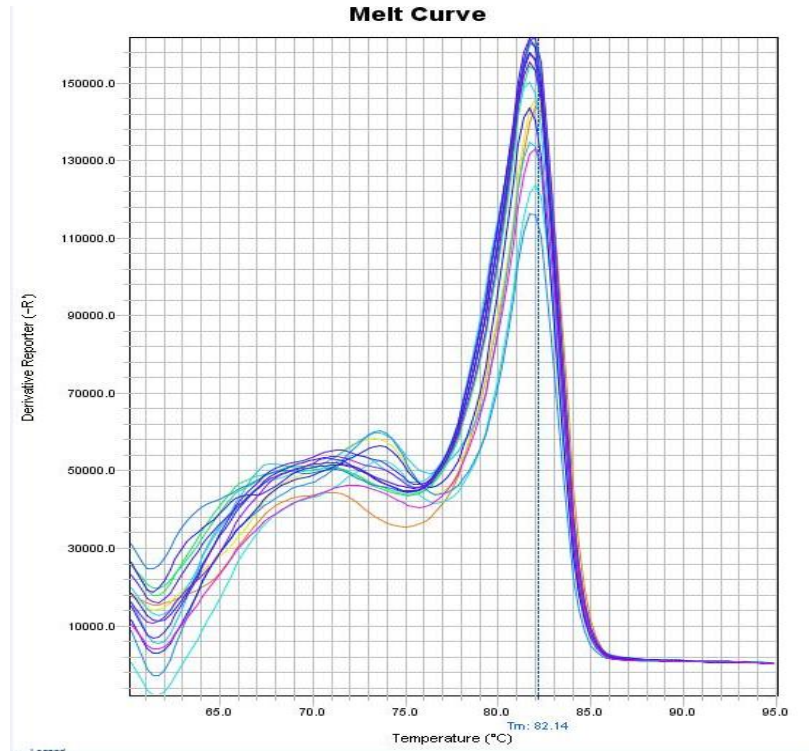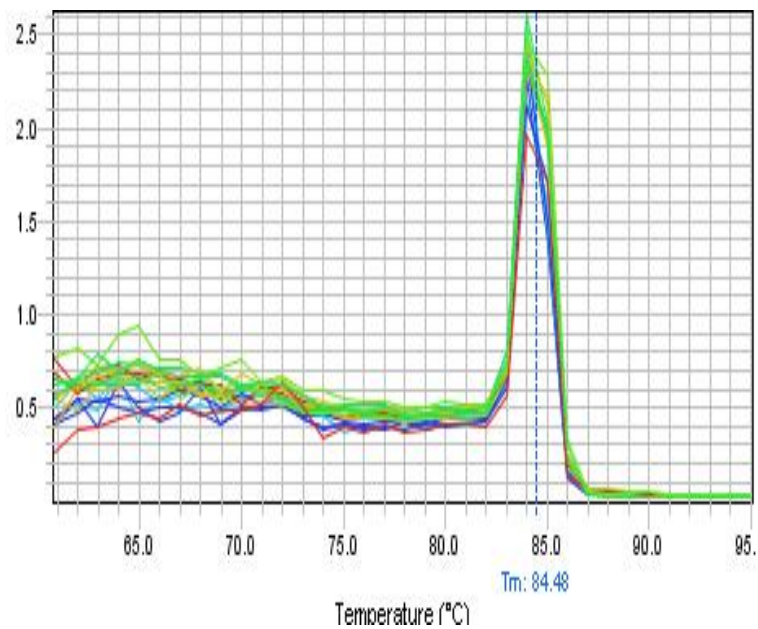

Fig. 4. The melt curve of the GAPDH
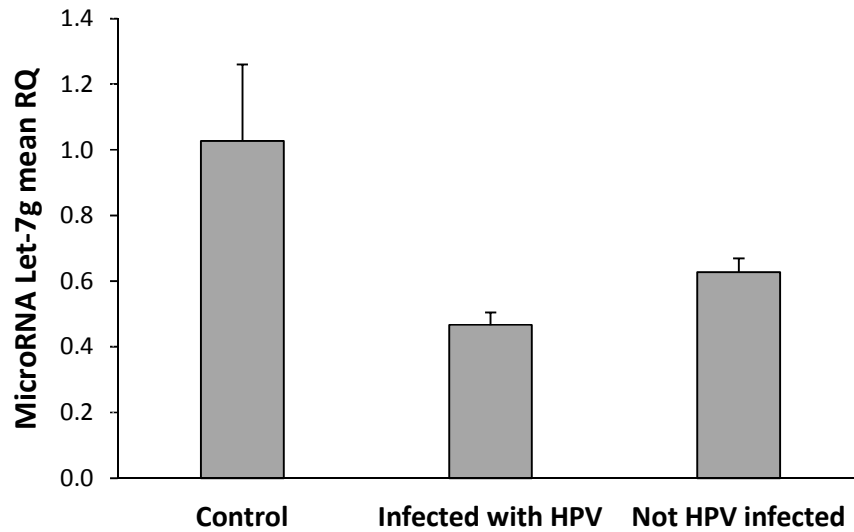

Fig. 5. The melt curve of the HMGA$_2$

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Fig. 6. The miR Let-7g expression in control group, HPV infected and not infected patients
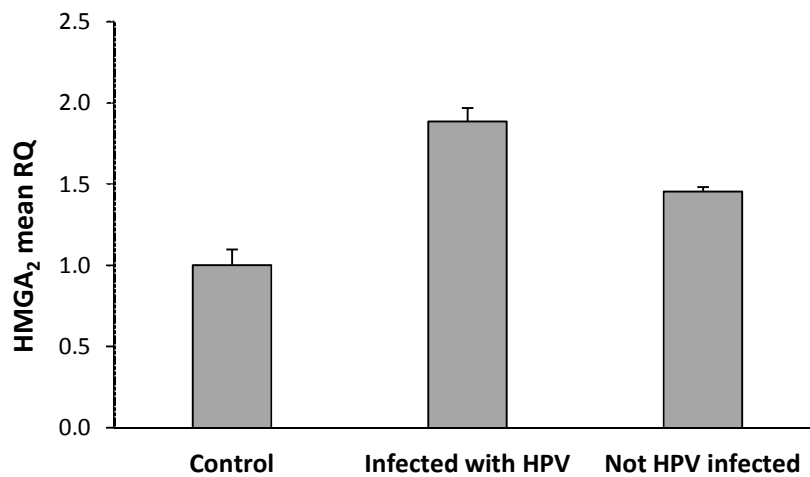

Fig. 7. The HMGA$_2$ gene expression in control group, HPV infected and not infected patients

MiR Let-7d is one of the members of the human lethal-7 family and its level is reported to decrease in many types of cancers [22]. Similar to the microRNAs, the Let-7 expression down regulates in the lung, gastric and colon cancers [10]. Re-expression of the Let-7g suppresses hepatocellular carcinoma tumorigenesis at the earlier stage [14]. Let-7d plays a key role in development and function of the female reproductive tract [23]. For instance, it seems that the Let-7 family may be essential in the developing ovarian cancer [24]. Based on the results, Let-7g expression decreased in HPV-infected compared to not infected cervical cancer patients as well as healthy individuals. It is reported that miR Let-7 reduces the proliferation and migration of glioblastoma cell lines which suppresses the xenograft tumours in mice [8]. The overexpression of Let-7 decreased cell invasion, proliferation, and increase apoptosis of human glioblastoma cells [8]. Let-7 known as a tumour suppressor in human lung and colon cancers, although Let-7a/i is dysregulated in gene profiling, it remains unclear whether it plays important role in endometrial carcinoma, [7].

The HMGA$_2$ is essential to regulate and mediate Let-7 action on the cell cycle progression and enhancing a stem cell phenotype (Madison et al. 2015). The cellular mechanism for reports is that the Let-7 has the ability to bind the terminal loops of precursors and blocking mature MicroRNAs processing [25]. Therefore, Let-7 has a key role as therapeutic agent for cancer treatment [8].

The let-7 family is located in fragile sites of the human genome. The HMGA$_2$, an oncogene over expressed in several tumours [26]. As seen, in the current study, the HMGA$_2$ gene expression significantly increased in cervical cancer which was in agreement to the previous reports [11; 27; 26]. Since HMGA proteins for the first time isolated in rat thyroid cells transformed by retroviruses, it is assumed HMGA proteins are strongly related to cancer because their level of expression increase in cancer compared to the normal cells [11]. They are flexible molecules which can modulate regulation of several gene expressions with their different domains [11]. Elevated HMGA$_2$ gene expression is reported in a number of tumours [28-29]. Correlation reported between HMGA$_2$ and Let-7 expressions showed a decrease in leiomyomas and there was an inverse correlation between Let-7 expression and its proposed target HMGA$_2$ gene [30]. Oncogenes and their signalling pathways are a potential connection between the level of expression of Let-7 and the biological characteristics of tumour cells [10]. The HMGA$_2$ is one of the important oncogenes in tumorigenesis, proliferation and invasion and which are targeted by Let-7 [10].

The mechanism of how Let-7 family regulated the expression of the other gens is still not clear, but it is probably connected with regulation and transcription of various genes [31]. Evidence suggests restoration of Let-7 expression has an anti-proliferative effect on cancer cells [14]. It is assumed oncogenes like HMGA$_2$ up regulate the expression of their downstream target proteins, which directly regulate the cell cycle, apoptosis and cell adhesion [10]. Perhaps, Let-7 acts as a tumour suppressor by the reserve of these oncogenic signalling pathways [10]. The Let-7 acts via binding to the 3'UTR of target mRNAs to regulate their expression [29]. There are negative-acting regulatory elements within the 3'UTR regulated the HMGA expression. The over expression of HMGA observes because of un-ability of this element to bind to the 3'UTR of HMGA [32]. In some cases, elevated HMGA levels can be located in microenvironments with

normal levels of the Let-7. This happens when the truncated 3'UTR region of the HMGA$_2$ mRNA lacks the Let-7 binding sites [26].

Presumably, Let-7 impresses its effect via the same binding element of HMGA$_2$ gene expression. For instance, Let-7 expression is lower in patients with a high HMGA expression in gastric cancer [33] and the HMGA$_2$ expression down regulated when Let-7 was overexpressed in uterine leiomyosarcoma [34]. So, it is suggested the Let-7 mediates the expression of the oncogene HMGA$_2$ via binding to its 3'UTR [35]. Let-7 alone or together with other genes may be used for cancer profiling and serve as a diagnostic marker. Changes in Let-7 along with the other gens still a marker of cancer transformation. The use of miRNA seems to be a more sensitive tool than the currently used histopathological methods [22]. In the current study, we found Let-7 level decreased while HMGA$_2$ expression increased in HPV-infected cervical cancer patients. It seems the observed results mediate via the mechanisms previously reported and molecular and cellular researches needed to determine the direct mechanism(s) of action for observed results.

5. CONCLUSION

In conclusion, these results suggested elevated decreased miR Let-7g expression might increase HMGA$_2$ gene expression. So, determining of the Let-7g and HMGA$_2$ levels might useful indicator for prognosis of the HPV-infected cervical cancer. This was the first attempt to study miR Let-7g and HMGA$_2$ gene expression in cervical cancer among Iranian patients. Larger population studies are required for a more accurate understanding of miR Let-7g and HMGA$_2$ gene expression.

CONSENT

As per international standard or university standard, the patient's written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Lee K, Lee AY, Kwon YK, Kwon H. Suppression of HPV E6 and E7 expression by BAF53 depletion in cervical cancer cells. Biochem Biophys Res Commun, 2011;412(2):328-33.

2. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. Inter J Cancer. 2015; 136(5):E359-86.

3. Pedroza-Torres A, Fernández-Retana J, Peralta-Zaragoza O, Jacobo-Herrera N, de Leon DC, Cerna- Cortés JF, et al. A microRNA expression signature for clinical response in locally advanced cervical cancer. Gynecologic Oncology. 2016; 142(3):557-65.

4. Zang B, Huang G, Wang X, Zheng S. HPV-16 E6 promotes cell growth of esophageal cancer via downregulation of miR-125b and activation of Wnt/β-catenin signaling pathway. Int J Clin Exp Pathol. 2015;8(10):13687-94.

5. Pasquinelli AE. The primary target of let-7 microRNA. Biochem Soc Trans. 2013; 41(4):821-4.

6. Díaz-González SM, Deas J, Benítez-Boijseauneau O, Gómez-Cerón C, Bermúdez-Morales VH, Rodríguez-Dorantes M, et al. Utility of MicroRNAs and siRNAs in Cervical Carcinogenesis. Biomed Res Int. 2015;2015:374924.

7. Liu P, Qi M, Ma C, Lao G, Liu Y, Liu Y, Liu Y. Let7a inhibits the growth of endometrial carcinoma cells by targeting Aurora-B. FEBS Lett. 2013;587(16):2523-9.

8. Wang G, Wang J, Zhao H, Wang J, Tony To SS. The role of Myc and let-7a in glioblastoma, glucose metabolism and response to therapy. Arch of Biochem and Biophys. 2015;580:84-92.

9. Zhang L, Huang J, Yang N, Greshock J, Megraw MS, Giannakakis A, et al. microRNAs exhibit high frequency genomic alterations in human cancer. Proc Natl Acad Sci USA. 2006;103(24): 9136-41.

10. Wang X, Cao L, Wang Y, Wang X, Liu N, You Y. Regulation of let-7 and its target oncogenes (Review). Oncol Lett. 2012; 3(5):955-60.

11. Sgarra R, Rustighi A, Tessari MA, Di Bernardo J, Altamura S, Fusco A, et al. Nuclear phosphoproteins HMGA and their relationship with chromatin structure and cancer. FEBS Lett. 2004; 574(1-3):1-8.

12. Madison BB, Jeganathan AN, Mizuno R, Winslow MM, Castells A, Cuatrecasas M, et al. Let-7 Represses Carcinogenesis and a Stem Cell Phenotype in the Intestine via Regulation of Hmga2. PLoS Genet. 2015; 11(8):e1005408.

13. Sun X, Fan C, Du N, Ren H. Possible carcinogenesis of tumor suppressor let-7. Med Hypotheses. 2013;81(3):410-3.

14. Chen K, Hou Y, Wang K, Li J, Xia Y, Yang X, et al. Re-expression of Let-7g microRNA inhibits the proliferation and migration via K-Ras/HMGA2/Snail axis in hepatocellular carcinoma. Biomed Res Int. 2014;2014:742417.

15. Chen X, Liang H, Guan D, Wang C, Hu X, Cui L, et al. A combination of let-7d, let-7g and let-7i serves as a stable reference for normalization of serum microRNAs. PLoS One. 2013;8(1):e79652.

16. Castle PE, Solomon D, Schiffman M, Wheeler CM. Human papillomavirus type 16 infections and 2-year absolute risk of cervical precancer in women with equivocal or mild cytologic abnormalities. J Natl Cancer Inst. 2005;97:1066-71.

17. Kaka GHR, Tiraihi T, Kheradmand JA, Azizzadeh Delshad AR. Study on in-vitro transdifferentiation of rat bone marrow stromal cells into neuroepithelial-like cells. IRCMJ 2009;11(2):133-9.

18. Jiang L, Mao P, Song L, Wu J, Huang J, Lin C, et al. miR-182 as a prognostic marker for glioma progression and patient survival. Am J Pathol. 2010;177(1):29-38.

19. Zhao J, Lu Q, Zhu J, Fu J, Chen YX. Prognostic value of miR-96 in patients with acute myeloid leukemia. Diagn Pathol. 2014;9:76.

20. Twiggs LB, Hopkins M. High-risk HPV DNA testing and HPV-16/18 genotyping: What is the clinical application? J Low Genit Tract Dis. 2011;15(3):224-30.

21. Boulet GA, Horvath CA, Berghmans S, Bogers J. Human papillomavirus in cervical cancer screening: Important role as biomarker. Cancer Epidemiol Biomarkers Prev. 2008;17(4):810-7.

22. Kolenda T, Przybyła W, Teresiak A, Mackiewicz A, Lamperska KM. The mystery of let-7d- a small RNA with great power. Contemp Oncol (Pozn). 2014; 18(5):293-301.

23. Lei L, Jin S, Gonzalez G, Behringer RR, Woodruff TK. The regulatory role of Dicer in folliculogenesis in mice. Mol Cell Endocrinol. 2010;315(1-2):63-73.

24. Childs A, Kinnell HL, He J, Anderson RA. LIN28 is selectively expressed in primordial and pre-meiotic germ cells in the human fetal ovary. Stem Cells Dev. 2012; 21(13):2343-9.

25. Heine VM, Priller M, Ling J, Rowitch DH, Schuller U. Dexamethasone destabilizes Nmyc to inhibit the growth of hedgehog-associated medulloblastoma. Cancer Res. 2010;70(13):5220-5.

26. Schoof CR, Botelho EL, Izzotti A, Vasques LR. MicroRNAs in cancer treatment and prognosis. Am J Cancer Res. 2012; 2(4):414-33.

27. Meyer B, Loeschke S, Schultze A, Weigel T, Sandkamp M, Goldmann T, Vollmer E and Bullerdiek J. HMGA2 overexpression in non-small cell lung cancer. Mol Carcinog. 2007;46(7):503-11.

28. Sarhadi VK, Wikman H, Salmenkivi K, Kuosma E, Sioris T, Salo J, et al. Increased expression of high mobility group A proteins in lung cancer. J Pathol. 2006;209(2):206-12.

29. Belge G, Meyer A, Klemke M, Burchardt K, Stern C, Wosniok W, et al. Upregulation of HMGA2 in thyroid carcinomas: A novel molecular marker to distinguish between benign and malignant follicular neoplasias. Genes Chromosomes Cancer. 2008; 47(1):56-63.

30. Wang T, Zhang X, Obijuru L, Laser J, Aris V, Lee P, et al. A micro-RNA signature associated with race, tumor size and target gene activity in human uterine leiomyomas. Genes Chromosomes Cancer. 2007;46(4):336-47.

31. Jiao LR, Frampton AE, Jacob J, Pellegrino L, Krell J, Giamas G, et al. MicroRNAs targeting oncogenes are down-regulated in pancreatic malignant transformation from benign tumors. PLoS One. 2012;7(2): e32068.

32. Borrmann L, Wilkening S, Bullerdiek J. The expression of HMGA genes is regulated by their 3'UTR. Oncogene. 2001;20(33): 4537-41.

33. Motoyama K, Inoue H, Nakamura Y, Uetake H, Sugihara K, Mori M. Clinical significance of high mobility group A_2 in human gastric cancer and its relationship to let-7 microRNA family. Clin Cancer Res. 2008;14(8):2334-40.

34. Shi G, Perle MA, Mittal K, Chen H, Zou X, Narita M, et al. Let-7 repression leads to HMGA2 overexpression in uterine leiomyosarcoma. J Cell Mol Med. 2009; 13(9B):3898-905.

35. Mayr C, Hemann MT, Bartel DP. Disrupting the pairing between let-7 and Hmga2 enhances oncogenic transformation. Science. 2007;315(5818): 1576-9.

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