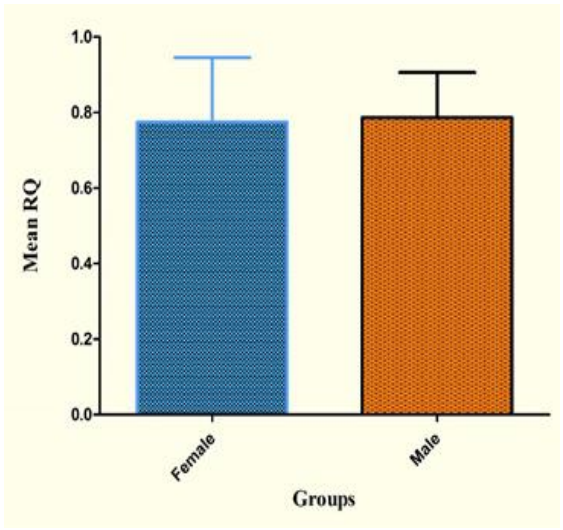

Fig 8. *Analysis of expression rate of Reprimo gene among sick cases based upon their gender ($p \leq 0.960$))*

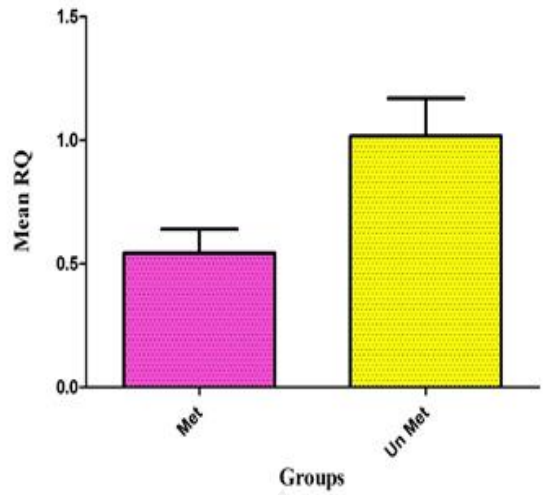

Fig 10. *Mean RQ of Reprimo gene based upon methylation status*

of 72°C for elongation, and a 1 final cycle of exposure to a temperature of 72°C for 5 minutes. Finally, 1.5% agarose gel was used to study the results of MS-PCR reaction.

Results and Discussion

The results of the expression rate of Reprimo gene

Ct of samples was calculated by the machine following the proliferation reaction and it was converted to RQ (Quantification Relative) of expression rate. Graph of the results was drawn by Graph Pad Prism software (Fig. 5).

The majority of gastric patients exhibited a reduced Reprimo gene expression rate compared to normal cases. In this case, the average of normal samples was set to 1 by machine analysis. As it was expected from Reprimo Gene as a suppressive tumor in gastric cancer, the expression rate of this gene in normal samples was much more than what was observed in the majority of samples suffering with gastric cancer. This correlation is significant as P-value < 0.05. The average of expression of gastric patients is much less than what was observed in normal samples. This comparison is a significant correlation considering the p-value (Fig. 10). An analysis

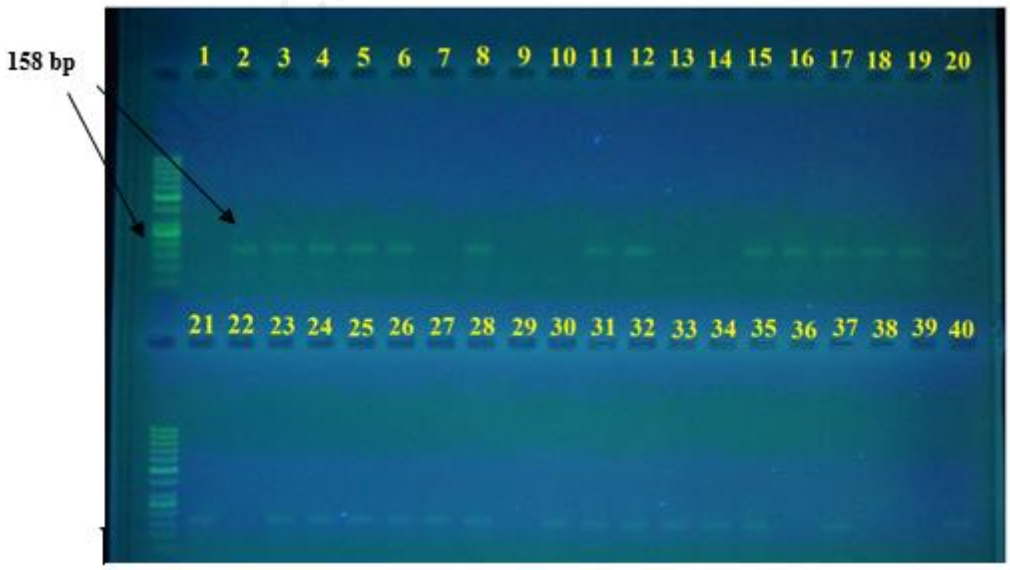

Fig 9. *Example of MS-PCR results obtained using methylated primers. The samples with a band size of 158 bp have methylation in the region of Reprimo gene promoter*

of results revealed that the expression rate of Reprimo gene among sick cases compared to healthy people (normal samples) younger than 55 is 1.206 while this value among those older than 55 was 0.673. This classification is solely based upon people's age group. As it is evident in chart 3, the mean expression rate of patients younger than 55 is statistically significant (Fig. 7). The expression rate of Reprimo gene among male patients was nearly equal to what was observed among female patients and the mean RQ level of men and women was 0.785 and 0.774 respectively (Fig 8). Considering chart 4, we may conclude that gender has no influence on the expression rate of Reprimo and pathogenicity of gastric cancer.

The results of MS-PCR using methylated primers

Having carried out MS-PCR using methylated primers on 100 intended samples in order to study the methylation status of Reprimo gene promoter, it turned out that 23 samples out of the total 50 DNA samples of patients were methylated, but this number in normal cases was only 7. As a result, 45.7% of samples for Reprimo gene promoter are methylated (Fig. 9). The expression rate of Reprimo gene in non-methylated samples turned out to be 99.6% while this value in methylated samples was 54%. The mean expression rate of Reprimo gene in non-methylated samples is more than what was observed in methylated samples.

Although recognition of factors such as helicobacter pylori and various environmental factors has helped reduce the prevalence of gastric cancer, it is still one of the most common types of cancers in the world and constitutes a major clinical challenge.[9] Gastric cancer is one of the most important causes of mortality in developing countries.[10] As a result of ineffectiveness of most common treatments, the majority of patients have a low survival rate even after treatment and pass away. Various studies have pointed to the fact that various factors such as helicobacter pylori infection and its resulting side effects and other factors such as genetics and its resulting polymorphism may have a major influence on sensitivity and development of gastric cancer.[11] The multi-factor nature of gastric cancer makes it impossible to come up with an accurate prohibition strategy and to diagnose it quickly. As the majority of gastric cancer cases have no symptoms until they reach the advanced level, early diagnosis of gastric cancer is very difficult.[12] The first documented epigenetic change in gastric cancer is hyper-methylation of the promoter of genes that restore inconsistent nucleotide mutations of DNA.[13] Several genes have been described as tumor repressors deactivated as a result of hyper-methylation in gastric cancer. Although recent reports point to the discovery of methylation of particular genes, no comprehensive characteristic of DNA methylation in gastric cancer has been reported to this date. Bernal et al started a research to find a gene with inappropriate hyper-methylation which is useful for early diagnosis.[15] By searching CpG islands in the promoter 24 region of candidated gene among 32 cases suffering with gastric cancer, they arrived at the conclusion that inappropriate methylation of Reprimo can act as a potential biomarker for early diagnosis of gastric cancer. Reprimo is the new candidate mediating termination of cell cycle in G2 phase with the aid of P53. Takao Takahashi et al. have pointed to the fact that Reprimo gene is not expressed by its promoter's methylation in pancreatic and lung cancer. They proved that hyper-methylation is responsible for transcription of Reprimo through hyper-methylation (92% match). Methylation of Reprimo promoter has been reported in 79% of gastric cancer, 62% of gallbladder cancer, 57% of lymphoma, 56% of clone cancers, 40% of esophageal cancer, 37% of breast cancer, and 31% of leukemia cases.[16] By studying 39 patients with an average age of older than 64 and a men to woman ration of 1.3 to 1 and comparing them against normal control samples, Alejandro et al arrived at the conclusion that Reprimo is superior in diagnosing non-invasive genes than CEA and CA 19_9. Sensitivity, specificity, positive predictive value, negative predictive value, and the rate of positive possibilities may be a great progress in assessing the diagnostic effect of Reprimo for noninvasive diagnosis of gastric cancer.[17] Considering the important effect of Reprimo gene in preventing gastric cancer and as a repressor in early diagnosis of gastric cancer among those suffering with it, we decided to study the expression rate of this gene in the blood sample of those suffering with gastric cancer in Iran. This project is the first comprehensive research studying the expression rate of gene in the blood samples obtained from those suffering with gastric cancer in Iran. Using Real Time PCR based upon cyber-green of RPRM gene expression and utilizing MS-PCR technique, the methylation status of Reprimo gene promoter was studied. As the results of this research showed, the expression rate of RPRM gene in the blood samples obtained from patients is much less than what is observed in normal blood samples. Considering that all patients with gastric cancer who participated in this study were in the early stages of cancer, based on our results, the study of Reprimo gene hypermethylation can be useful in early diagnosis of gastric cancer. Our results are in line with those reported by Kathleen Saavedra et al. pointing that Reprimo gene is a tumor repressor and its methylation in the blood samples obtained from those suffering with gastric cancer is much higer than what we see in healthy individuals (45.7% of patients constituting a significant correlation). It can also act as a biomarker for early diagnosis of gastric cancer. There is also a significant correlation between expression rate of Reprimo and the age of people suffering with cancer and the methylation status of its promoter. On the hoter hand, the correlation between the expression rate of Reprimo gene and the gender of those suffering with cancer was far from being significant.

List of acronyms

MMULV - Moloney Murine Leukemia Virus
MS-PCR - Methylation Specific-PCR
RPRM - Reprimo, TP53 Dependent G2 Arrest Mediator Homolog
RQ - Quantification Relative

Author's contributions

Each author contributed in equal part to the manuscript.

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Conflict of Interest

The authors declare no conflicts of interests.

Ethical Publication Statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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