Research Article

**ptk2 and mt2a Genes Expression in Gastritis and Gastric Cancer Patients with Helicobacter pylori Infection**

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**Background.** ptk2 and mt2a genes contribute to the cell cycle during proliferation and apoptosis, respectively. Designing a case-control study including gastric adenocarcinoma and gastritis patients with and without *Helicobacter pylori* infection would lead to determination of the correlations between ptk2 and mt2a genes expression with *H. pylori* infection in gastric antral epithelial cells.

**Methods.** Overall, 50 and 30 gastric antral biopsy samples of gastric cancer (case group) and gastritis (control group) patients were included into study, respectively. All biopsy samples were collected considering the exclusion criteria including patients with a history of consumption of tobacco, alcohol, and anti-*H. pylori* drugs. Each patient group is divided into with and without *H. pylori* infection to detect cDNA fold changes of ptk2 and mt2a genes by using Real Time RT PCR. Furthermore, the presence of *H. pylori* virulence genes was detected directly by using specific primers and simple PCR on cDNA synthesized from total RNA of gastric antral biopsy samples.

**Results.** A negative correlation was revealed between age and clinical manifestations with the ΔCt value of the ptk2 gene (*P* < 0.05). The *H. pylori* iceA1/2 and cagE genes revealed positive and negative correlations with the ΔCt value of the ptk2 gene (*P* < 0.05), respectively. Furthermore, a weak correlation was detectable between *H. pylori* babA2/B, oipA, and cagY genes and the ΔCt value of the mt2a gene in gastric antral epithelial cells of patients (*P* < 0.1).

**Conclusions.** The results of the current study opened a view for more investigation on the stunning roles of *H. pylori* infection in clinical outcomes through mt2a and ptk2 gene expression in gastric antral epithelial cells.

1. **Introduction**

In a close competition between carcinogenic agents and natural anti-cancer drugs such as *Allium*, cell signaling molecules are considered the effective targets [1]. In this regard, it has been revealed that the main pathways of natural anticancer drugs are to induce apoptosis by activating the MAPK and PI3K/AKT signaling pathways suppressing tumor growth [1]. In this meantime, Mt2a (Metallothionein 2A) as a member of the metallothionein proteins family contributes to critical roles including increasing intracellular concentration of heavy metals, protecting the cells against the toxicity of hydroxyl free radicals and heavy metals affecting cell apoptosis, proliferation, angiogenesis, and anti-inflammatory processes [2–4]. As a critical role in cell cycling, Metallothioneins control HMBOX1 (Homeobox containing 1) protein bound to the double-stranded repeat sequence of telomeres following regulation of zinc intracellular concentration [5–8]. Recent studies have shown that following LPS-induced inflammation and ROS production, HMBOX1 inhibits NF-κB and the MAPK signaling pathway [1, 9]. In this regard, MT2A protein exerts its anti-cancer effects through inactivation of NFKBIA (NFkB Inhibitor Alpha) subsequent binding to
MZF1 (Myeloid Zinc Finger 1) [9, 10]. As a clinical impact of Mt2a, it has recently been shown that attenuation of MT2A and Ikb-α gene expression in human gastric epithelial cells is associated with a poor prognosis in gastric cancer patients [9, 10]. Furthermore, some mt2a gene polymorphisms, including rs28366003 and rs1610216, are associated with an increased risk of cancer [11–13]. In this regard, some MT-2A gene polymorphisms are associated with adenocarcinoma risk in the Iranian patient population [14]. Moreover, in the Polish population, the rs28366003 SNP polymorphism has been associated with breast and prostate cancer [15].

On the other hand, the ptk2 gene encodes a cytoplasmic tyrosine kinase protein, namely, FAK (Focal Adhesion Kinase) [16–18]. The FAK/Paxillin signaling pathway conducts a substantial effect on the cell migration following activating SH2 domains of the Src kinase family and consequently triggering downstream-activating signals leading to the regulation of cell motility, invasion, survival, and proliferation [19]. The changes in ptk2 gene expression are associated with the pathological stage, progression, and cancer’s specific survival [20]. There is evidence to support the role of FAK in promoting malignant phenotypes (unregulated cell proliferation, survival, and migration) of various tumor cells in vitro [21, 22]. The ptk2 gene over-expression is commonly seen in metastatic cancers and is obviously associated with malignant clinical outcomes [23–25]. Furthermore, inhibitors of FAK kinase exert suppressing cancer cell growth in clinical trials [26–28]. In the other studies, the ptk2 gene over-expression is well-known as a pathological risk factor predicting aggressive biological behavior of bladder carcinoma cells [29]. Considering a previous study, FAK is highly expressed in human gastric cancer cells and correlated with tumor progression, invasion, and metastasis [30]. To conclude, the studies show that the Fak protein and its gene [ptk2] are significantly associated with cancer progression [31, 32].

H. pylori as the most common causative agent of chronic infections in humans shows a full long history related to clinical implications from gastritis to gastric cancer [33]. In this regard, the studies show that the H. pylori virulence factors have an indispensable impact on the clinical consequences of H. pylori persistent active infection [33–37]. In the meantime, the epidemiological studies show that H. pylori adheres, including Sab, Bab, and the type 4 secretory system (T4SS), are related to peptic ulcers and gastric cancer [33–36]. CagA suppresses the apoptotic activity of VacA and activates the signaling pathway of the factor NF-KB inducing overexpression of anti-apoptotic agents [33]. On the other hand, CagY and CagL proteins, as the significant members of T4SS proteins with a tubular structure, following contact with gastric epithelial cell surface receptors, including beta-1 integrin receptors, inject CagA carcinogen protein into the gastric epithelial cell [37–40]. Therefore, it is worth to mention that despite the presence of the cagA gene in the H. pylori strains genome, due to possible changes in the cagA gene expression under the full stress environment of the stomach, the CagA protein could not be able to inject into host cells [39]. This study investigated the correlation between the H. pylori virulence genes and ptk2 and mt2a genes expression in gastric antral epithelial cells of gastritis and gastric cancer patients with H. pylori infection.

2. Materials and Methods

2.1. Sampling. A case-control study was designed on patients who had involved gastric adenocarcinoma and gastritis [41, 42]. Considering the prevalence of gastric adenocarcinoma and H. pylori infection in Sanandaj city, located in the west of Iran, using Cochran’s formula, 30 and 50 patients with gastric adenocarcinoma and gastritis were registered [42]. Patients with a history of anti-H. pylori chemotherapy, alcohol, and cigarette consumption were excluded from the study. According to the study’s aim, to investigate H. pylori infection’s effect on the mt2a and ptk2 genes expression, the gastric biopsy samples were collected from patients referred to Tohid Hospital in Sanandaj city.

The H. pylori active infection was detected by using a urea breath test [43, 44]. Two gastric biopsy specimens were obtained from each patient by a gastroenterologist. One biopsy sample was prepared from the gastric antrum, the natural site of H. pylori infection, to detect H. pylori virulence genes and study mt2a and ptk2 gene expression using PCR, and another biopsy from the gastric adenocarcinoma tumor area to pathologic evaluation. The gastric antral biopsy sample for molecular analysis was immediately dropped into RNA Later solution and transferred to the Molecular Microbiology Laboratory of Kurdistan University of Medical Sciences. On the other hand, the gastric tumor area biopsy samples including cardia, body, or pyloric area, were evaluated at the pathology center of Tohid Hospital.

2.2. PCR. First of all, total RNA was extracted from gastric antral biopsy samples (Miniprep Kit; Bio Basic Company, Toronto, Canada) and immediately converted to cDNA (Prime Script™ RT-PCR Kit; Takara Company, Japan). To survey the presence of the H. pylori genome in gastric antral biopsy samples, H. pylori 16s rRNA gene-specific primer and PCR method were used. Primers of H. pylori virulence genes were designed by using primer3 online software and blast on the PubMed website and then were synthesized by Bioneer company (South Korea; Tables 1 and 2). The thermocycler program was set based on the current protocols. Using Takara kits (Emerald Amp® MAX PCR Master Mix), PCR master mix was prepared. Single PCR steps were performed according to the current PCR protocols and using annealing temperatures of primers in a final volume of 25 microliter (Eppendorf® Co.; Tables 1 and 2) (17). Based on agarose gel electrophoresis protocols, PCR products were run on 1% agarose gel to observe PCR results. To prove the H. pylori genome, the H. pylori vacA m2 gene was sequenced and registered in the GenBank with accession number MK642592.1.

2.3. Real-Time RT PCR. To detect mt2a and ptk2 genes’ expression in the gastric biopsy samples by using the Real-Time RT PCR method (Corbett machine, Co.), the specific
### Table 1: Primers of *H. pylori* T4SS, 16s rDNA, and vaccA in simple PCR.

| Specific primers | Sequence                  | Annealing Tm°C | Product size bp | Reference          |
|------------------|---------------------------|----------------|-----------------|--------------------|
| 16s rDNA H. pylori F | CTGGAGAGACTAAGCCTCC        | 50             | 446             | This study         |
| 16s rDNA H. pylori R | AGGATCAAGGTTTAAGGATT       |                |                 |                    |
| cagA F          | TGACCAACACACGACACGAGC     | 57             | 108             | This study         |
| cagA R          | AGGATCAAGGTTTAAGGATT       |                |                 |                    |
| cagA EPIYA-C F  | AAGAAAGGACAGAACACCAACG    | 55             | 188             | This study         |
| cagA EPIYA-C R  | CTACCAATGGCCTGACACGT      |                |                 |                    |
| cagF F          | AGGGATTGATGATAGACG        | 55             | 154             | This study         |
| cagF R          | TGCTTCTTCTTCTGACTC        |                |                 |                    |
| cagF E          | AGATGAGAGGAGGAGGAGC       | 56             | 163             | This study         |
| cagF R          | TAGGAATTTGGACGCTCAC        |                |                 |                    |
| vacA F          | AGTTCAAGGAGGAGGAGGAGC     | 57             | 200             | This study         |
| vacA R          | ACAAGGCTTTCAAGGATCGTG      |                |                 |                    |
| vacA 1/s2 F     | ATGGATAATACACAAACACAC     | 55             | 259/286*        | Atherton et al. [46]|
| vacA m1/m2 F    | TGAGATGAGAGGAGGAGGAGC     | 54             | 205/220*        | This study         |
| vacA m1/m2 R    | TAGGAATTTGGACGCTCAC        |                |                 |                    |

*The primers yield various fragments of vaccA depending on the presence of a repetitive nucleotides sequence in some vaccA alleles.*

### Table 2: Primers of *H. pylori* adhesins and surface proteins.

| Specific primers | Sequence                  | Annealing Tm°C | Product size bp | Reference          |
|------------------|---------------------------|----------------|-----------------|--------------------|
| iceA1 F          | GTTGTATTTTAACTACAAATC     | 45             | 247             | Van Doorn et al. [47]|
| iceA1 R          | GTTGTATTTTAACTACAAATC     |                |                 |                    |
| iceA2 F          | GAGCAAGGAGAAACGATG       | 47             | 229/234         | Van Doorn et al. [47]|
| iceA2 R          | GAGCAAGGAGAAACGATG       |                |                 |                    |
| hopQI F          | AGAAGCCACTCATCCCGAGA     | 55             | 187             | Sicinschi et al. [48]|
| hopQI R          | AGAAGCCACTCATCCCGAGA     |                |                 |                    |
| hopQII F         | ACACCACTCATCCCGAGA       | 55             | 160             | Sicinschi et al. [48]|
| hopQII R         | ACACCACTCATCCCGAGA       |                |                 |                    |
| babA2 F          | CAAATCGGAGGATCGAAAAAC    | 57             | 205             | This study         |
| babA2 R          | CAAATCGGAGGATCGAAAAAC    |                |                 |                    |
| babB F           | CAAATCGGAGGATCGAAAAAC    | 56             | 175             | This study         |
| babB R           | CAAATCGGAGGATCGAAAAAC    |                |                 |                    |
| sabA F           | TGGGTCTGTTGCTGATCATC    | 56             | 204             | This study         |
| sabA R           | TGGGTCTGTTGCTGATCATC    |                |                 |                    |
| sabB F           | TGCCTGCTGTTGCTGATTCC    | 56             | 248             | This study         |
| sabB R           | TGCCTGCTGTTGCTGATTCC    |                |                 |                    |
| alpA F           | CTGCCTCTCAAAACCCGCTC   | 55             | 185             | This study         |
| alpA R           | CTGCCTCTCAAAACCCGCTC   |                |                 |                    |
| alpB F           | TGGGTCTGTTGCTGATTCC    | 57             | 218             | This study         |
| alpB R           | TGGGTCTGTTGCTGATTCC    |                |                 |                    |
| oipA F           | CCGATCCGCTGAAAGGATG    | 55             | 233             | This study         |
| oipA R           | CCGATCCGCTGAAAGGATG    |                |                 |                    |

### Table 3: Specific Primers of ptk2 and mt2a in Real-Time RT PCR.

| Specific primers | Sequence                  | Annealing Tm°C | Product size bp | Reference          |
|------------------|---------------------------|----------------|-----------------|--------------------|
| GAPDH F          | GAAAGTTGAAAGTGGTCAAAGTAC  | 60             | 71              |                    |
| GAPDH R          | CAGAGTTAAAGAGACGCTCGT    |                |                 |                    |
| ptk2 F           | GACCCACCGAGAGATTGAG      | 60             | 81              | [49]              |
| ptk2 R           | GAGCAGGTTTTGCATCGTTGA    |                |                 |                    |
| mt2a F           | GCCGACGGTGGATCTG         | 60             | 102             |                    |
| mt2a R           | TTTGTTGAAAGTGGTCTTTTA    |                |                 |                    |
primers of mt2a and ptk2 as the target genes and GAPDH as the internal genes were designed by using primer3 software and their sequences in the GenBank (Table 3). Takara kits (Emerald Amp® MAX PCR Master Mix) were used to prepare the PCR master mix. The PCR amplification program included a 10 minutes denaturation step at 95°C and 40 cycles of denaturation at 95°C for 15 seconds, specific annealing temperatures for 30 seconds, and extension at 72°C for 60 seconds. The relative quantity of the mt2a and ptk2 genes expression in gastritis (control group) and gastric adenocarcinoma (case group) patients were calculated by ΔΔCt (threshold cycle) = Ct (DNMT1) Ct (GAPDH). The mean of Ct of each 3 PCR reactions was put on the ΔΔCt formula to compute the fold change of each gene in the case and control groups by using the 2−ΔΔCt formula [43–45].

2.4. Statistical Analyses. Quantitative and qualitative data analysis was performed using SPSS software version 26. Crosstab chi-square tests were used to survey the correlation and relationship between qualitative data [41, 42]. First, the quantitative data distribution status was determined using Kolmogorov–Smirnov statistical test [42]. The distribution of mt2a and ptk2 genes ΔCt was abnormal. Accordingly, Mann–Whitney and Kruskal–Wallis tests were used to investigate the correlation between quantitative data and comparison in the case and control groups and subgroups [42]. The significance level was considered less than 0.05 [41].

3. Results

Based on the urea breath test results, 46% and 36.66% of gastritis and gastric adenocarcinoma patients showed H. pylori active infection, respectively (Table 4). It revealed that because of an anti-H. pylori drug without medical registration, the prevalence of H. pylori active infection among gastric adenocarcinoma patients was significantly lower than in gastritis patients (Table 4). Like in the previous studies, the frequency of H. pylori infection and gastric adenocarcinoma increased along with increasing age (P < 0.001; Tables 4 and 5).

Besides the genetic factors, exposure to environmental carcinogens factors, diet and H. pylori infection contribute to increasing gastric cancer risk [43, 44]. In this regard, although the results of the current study showed that the prevalence of gastric adenocarcinoma is significantly higher in men (P < 0.05; Table 4), it is worthy of note that this significant difference was not related to H. pylori infection (Table 5).

The changes of ptk2 and mt2a genes ΔCt in demographic subgroups indicated a negative correlation between ptk2 gene ΔCt with patients’ age and clinical manifestations (Table 6 and Figure 1). This means that the ptk2 gene expression in gastric antral epithelial cells increases with getting older and gastric cancer up 2.51 and 4.32 times, respectively (P < 0.005). In addition, a significant decrease of ΔCt of the ptk2 gene was revealed in patients with H. pylori cagЕ− strains infection (Table 7). In these cases, ptk2 gene expression increased 5.06-fold compared to cagЕ+ samples (P < 0.05; Table 8). On the other hand, gastric antral biopsies with infection of H. pylori iceA1/2+ strains showed a positive correlation with the ΔCt of the ptk2 gene (P < 0.005). In samples with iceA1/2+ cDNA, the expression level of the ptk2 gene increased by 7.66-fold than in samples with iceA2− cDNA (P < 0.005).

Although no correlation was observed between H. pylori infection and ΔCt of ptk2 and mt2a genes, a weak correlation was observed between mt2a gene ΔCt and subgroups with babA2/B, oipA, and cagY cDNA (P < 0.1; Table 9).
Figure 1: A simple scatter plot of ΔCt of the ptk2 gene depicts a reverse relationship with R2 linear 0.057. This means the ptk2 gene expression will increase along with increasing age.

Table 7: Correlations between ptk2 and mt2a ΔCt and gene expression of H. pylori virulence genes in gastric antral epithelial cells of gastritis and gastric adenocarcinoma patients.

| gene | vacA | s1m1/s1m2 | cagA | cagA- | cagT | cagY | cagE | sabA/B | babA2/B | hopQI/II | alpA/B | oipA | iceA1/2 |
|------|------|----------|------|-------|------|------|------|--------|---------|----------|--------|------|-------|
| ptk2 correlation | 0.320 | −0.216 | −0.156 | −0.082 | −0.238 | −0.390 | −0.215 | 0.192 | −0.192 | 0.186 | 0.152 | 0.519 |
| mt2a correlation | −0.256 | 0.292 | 0.304 | 0.009 | −0.378 | 0.289 | −0.367 | −0.388 | 0.040 | −0.093 | −0.348 | −0.293 |
| P value | 0.065 | 0.312 | 0.468 | 0.804 | 0.262 | 0.023 | 0.314 | 0.370 | 0.368 | 0.384 | 0.472 | 0.002 |
| P value | 0.227 | 0.166 | 0.149 | 0.966 | 0.068 | 0.171 | 0.087 | 0.061 | 0.854 | 0.665 | 0.095 | 0.165 |

Table 8: The statistical relationships of ptk2 genes expression with H. pylori infection and its virulence genes expression using Mann–Whitney U test. Fold changes of ptk2 gene expression were calculated by using the average of ptk2 ΔCt in the case and control groups (P < 0.05).

| Disease | N | Mean rank | Z | P value | Mean ΔCt | ptk2 | Fold change |
|---------|---|-----------|---|---------|----------|------|-------------|
| Adenocarcinoma | 30 | 29.20 | −3.371 | 0.001 | 5.4730 | 2.51 |
| Gastritis | 50 | 47.28 | 6.8048 | 0.001 |
| 61–85 | 28 | 18.48 | −3.163 | 0.002 | 5.6203 | 4.32 |
| Age group | 31–45 | 18 | 31.31 | 7.7354 | 0.001 |
| iceA1/2 | 17 | 12.4122.59 | −2.983 | 0.002 | 4.8719 | 7.66 |
| iceA2/iceA1/2 | 17 | 12.4122.59 | −2.983 | 0.002 | 7.8093 | 7.66 |
| cagE | Positive | 5 | 8.3 | −2.239 | 0.023 | 4.3460 | 5.06 |
| Negative | 29 | 19.09 | 6.6845 | 0.001 |

Table 9: The statistical results of mt2a gene expression with H. pylori infection and its virulence genes expression using Mann–Whitney U test. Fold changes of mt2a gene expression were calculated by using the average of mt2a ΔCt in the case and control groups (P < 0.01).

| gene | babA2/B | N | Mean rank | Z | P value | Mean ΔCt | mt2a | Fold change |
|------|-------|---|-----------|---|---------|----------|------|-------------|
| Negative | babA2/B | 17 | 3 | 9.08 | 3.67 | −1.876 | 0.061 | 5.3767 | 3.0167 | 5.14 |
| Negative | Positive | 3 | 1 | 20.50 | 11.77 | −1.671 | 0.095 | 6.3100 | 4.6918 | 2.71 |
| cagY | CagY-MRR | negative | 13 | 21 | 9.40 | 14.71 | −1.815 | 0.068 | 4.1490 | 5.2850 | 2.2 |

<image>
samples with babA2/B+ cDNA, the expression of the mt2a gene increased 5.14-fold than in samples with babA2/B- cDNA ($P < 0.005$; Table 9).

4. Discussion

MT2A, as an oxidative shock protein protecting cell DNA, influences the cell cycle through apoptosis [2–4]. Pan et al. showed that overexpression of the mt2a gene is associated with increasing tumor grade and poor gastric cancer prognosis [10]. They showed that MT2A induces apoptosis by stopping the G2/M phase of the cell cycle, and the effect of MT2A on apoptosis is due to the suppression of the NF-KB signaling pathway [9, 10]. Wang et al. showed that MT2A increases the expression of P21 and BAX proteins by affecting P53 which is a pre-apoptotic protein triggering the cell death process [12].

Previous studies show the positive correlations between mt2a gene polymorphisms and breast, liver, and prostate cancers [11–15]. In a comparison, the results of the current study showed no statistically significant correlation between mt2a gene $\Delta$Ct and patients’ demographic characteristics, including clinical manifestations, tumor grade, adenocarcinoma tumor area, age, sex, and H. pylori infection (Table 5). On the other hand, weak and negative correlations were observed between the cDNA of babA2/B, oipA, and cagY virulence genes of H. pylori with mt2a gene $\Delta$Ct ($P < 0.1$; Table 7). The mt2a gene expression in gastric biopsy samples with H. pylori babA2/B+ cDNA was higher up to 5.14-fold than in biopsy samples with H. pylori babA2/B- cDNA (Table 9). These results indicate that the simultaneous expression of H. pylori babA2+ and babB+ genes (babA2/B+ cDNA) correlates weakly with increased mt2a gene expression ($P < 0.1$). Furthermore, previous studies have shown the negative and positive correlations between gastric cancer with H. pylori babA2/B and cagE, cagA, and iceA1 genes expression, respectively (Table 9).

Aras et al. showed that the cagY gene has two reproducible and variable regions at the $5'$ end sequence including forward (FRR) and middle (MRR) [38]. They showed that following an increase or decrease in the copy number of reproducible sequences, the cagY gene expression and consequently CagY protein’s efficiency to bind to $\beta 1$ integrin receptors of gastric epithelial cells will change [38]. They revealed that changes in the CagY protein antigens would give a trait to bacteria to escape from the gastric mucosal immune system and establish a persistent infection [38]. The present study showed a statistically weak correlation between the H. pylori cagY gene with the MRR region and increased mt2a gene expression up to 2.2 times ($P < 0.1$; Table 9). The mt2a gene expression in the biopsy specimens with the H. pylori cagY-MRR+ cDNA was higher than in the biopsy specimens with the H. pylori cagY-MRR cDNA ($P < 0.1$). FAK protein is known as a tyrosine kinase integrin that its gene expression will increase along with cancer progression [19–22]. Some studies have shown a gradual increase in FAK protein (ptk2 gene) related to poor cancer prognosis [29]. Zhang et al. showed a high ptk2 gene expression correlated with aggressive traits of bladder squamous cell carcinoma [29]. Their Kaplan–Meyer analysis also showed a correlation between ptk2 gene expression and the pathological stages, invasion of the disease, and reduced chance of survival in carcinoma [29]. The present study showed that the ptk2 gene expression and the frequency of gastric cancer increase along with increasing age ($P < 0.001$).

Previous studies, based on the bacterial effects on the host cytoskeleton, show Hijacking of the FAK/Paxillin pathway to control cell physiological trends [51]. In this regard, Kim et al. showed following being attached Shigella flexneri OspE protein to integrin-binding kinase, the integrin B1 gene expression will be increasing, and suppression occurs as a result of phosphorylation of FAK and Paxillin [51].

5. Conclusion

In a remarkable conclusion, this study showed that the gastric antral epithelial samples with infection of H. pylori iceA1/2 and cagE genotypes reveal a strong correlation with increased ptk2 gene expression ($P < 0.001$).

Data Availability

All data are already released in the article results and the raw datasets used and/or analyzed during the current study are not publicly available due to contracts with research participants but are available from the corresponding author upon reasonable request.

Consent

The institutional review board has approved the study as no published patients’ names were involved in the research project.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors’ Contributions

Manouchehr Ahmadi Hedayati, the corresponding author, investigated and designed the study, Primers and Real-Time PCR; Delniya Khani was responsible for sampling, Real-Time PCR; Farshad Sheikhesmaeili: was responsible for sampling; and Bijan Nouri was responsible for statistical analysis.

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