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Research Article

PTK2 mRNA Expression in Bladder Cancer: Insights from Multi-Dimensional Analysis and Gene Interaction Networks

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Abstract

Background: PTK2 (focal adhesion kinase) is implicated in cancer progression and metastasis. This study was aimed to comprehensively analyze the PTK2 expression patterns, protein interactions, and its correlation with immune infiltration in bladder cancer to elucidate its clinical relevance.

Methods: Public databases including The Cancer Genome Atlas, UALCAN, STRING, and Tumor Immune Estimation Resource were evaluated. Differential PTK2 mRNA expression in bladder tumors versus normal tissues was evaluated. PTK2 correlations with cancer stage, gender, immune infiltrates, and integrin β1 expression were examined. Protein-protein interaction networks were generated using STRING.

Results: PTK2 was significantly overexpressed in bladder cancer, especially in higher-stage tumors. PTK2 positively correlated with B cells and macrophages but negatively correlated with CD4+ T cells and dendritic cells. Increased CD8+ T cell infiltration is associated with a better prognosis. Protein network analysis showed that PTK2 interacts with several proteins including INTB1, INTB3, PTEN, PI3K, CRK, PXN, VCL, BCAR1, GRB2, and SRC. Gene expression analysis revealed a positive correlation between PTK2 and INTB1 gens.

Conclusions: Bioinformatics analyses implicate PTK2 overexpression in bladder cancer progression and associations with immunosuppressive microenvironments. Findings suggest PTK2 may be a potential prognostic biomarker and therapeutic target warranting further investigation. More experimental validation is needed to confirm computational perspectives.

Keywords: PTK2; INTB1; Bladder Cancer

Introduction

Bladder cancer is the sixth most common cancer worldwide, with over 80,000 new cases in the US alone in 2022 [1,2]. The majority of bladder cancers originate from the inner lining of the bladder as urothelial carcinomas. While survival rates are relatively high for early-stage, non-muscle invasive tumors, prognosis drastically worsens once cancer invades the muscle wall [3-5]. Even early-stage tumors are prone to recurrence, requiring lifelong surveillance. Unfortunately, our understanding of the molecular events driving bladder carcinogenesis and progression remains incomplete [6]. Genome-wide profiling studies have uncovered some genetic alterations that occur in bladder cancer, such as mutations in FGFR3, TP53, PTK2, and the RTK/RAS/PI3K pathway [7-10]. However, the heterogeneity and complexity of the mutational landscape across different tumor stages and grades highlight our need for deeper, more integrated knowledge of the pathogenic mechanisms underlying this disease.

PTK2 (also known as FAK) is a non-receptor protein tyrosine kinase that localizes to focal adhesions and plays an important role in integrin-mediated signal transduction [11]. Extensive evidence shows the expression and activity of PTK2 are upregulated in various cancers, promoting cancer cell migration, invasion, and metastasis [12,13]. Beyond the effects of various genetic and protein alterations within tumor cells themselves, defects in immune system activation and recruitment to cancerous tissues also play a critical role. Recent studies have illuminated the impacts of specific proteins on anti-tumor immunity. In the complex landscape of cancer, PTK2 activation plays a pivotal role. Specifically, in melanoma, it triggers the increased secretion of chemokines such as CCL2, recruiting macrophages, and myeloid-derived suppressor cells into the tumor, thereby establishing an immunosuppressive microenvironment [14,15]. In contrast, in breast cancer, heightened PTK2 expression was linked to amplified intratumoral CD8+ T cell density, although these T cells displayed compromised cytotoxic function [16]. In our previous experimental study, we found increased expression of PTK2/FAK mRNA in bladder cancer tissues, especially in recurrent cancers compared to newly diagnosed ones. There was also a positive correlation between PTK2/FAK and activated β1 integrin, suggesting FAK is part of a mechanotransduction pathway promoting bladder cancer progression after initial surgery [17]. Furthermore, PTK2 knockdown inhibits bladder cancer progression in preclinical models, implicating its oncogenic role [18]. Despite evidence implicating PTK2 overexpression and hyperactivation in more aggressive bladder cancer phenotypes the underlying mechanisms remain unclear [9], further in-depth characterization of this signaling molecule may provide critical insights into the pathogenic processes underlying bladder cancer growth and progression. Comprehensively evaluating PTK2 expression through multi-omics bioinformatics approaches could shed light on its utility as a biomarker or therapeutic target for improved management of this disease.

In this study, we will perform an integrative bioinformatics analysis to comprehensively characterize PTK2 expression patterns, protein interactions, associated pathways and the relationship between PTK2 expression levels and immune cell infiltration in bladder cancer. The goal is to elucidate the oncogenic functions and clinical relevance of PTK2 as a potential prognostic biomarker and therapeutic target for improved management of bladder cancer patients.

Materials and Methods

Databases and gene expression analysis

The study employed datasets from publicly accessible databases, The Cancer Genome Atlas (TCGA) (https://cancergenome.nih.gov), the UALCAN database [19,20], and the Tumor Immune Estimation Resource (TIMER) [21,22]. Our primary objective was to assess the differential expression of PTK2 mRNA in healthy versus tumor tissues across various cancer types. The differential expression of PTK2 mRNA was also analyzed by gender and cancer stage, with the same fold change threshold of >1.5 and a gene rank within the top 10%. This study used only publicly available data and did not require ethical approval.

Tumor infiltration analysis

TIMER, using TCGA data, offers an estimation of immune cell infiltration in tumors by computing gene expression correlations between malignancies and immune cell types. We utilized Spearman's correlation coefficients to elucidate the associations between PTK2 expression and immune infiltrates. For clearer visualization and interpretation, data were transformed to log2 RSEM.

Correlation between PTK2 and ITGB1

Pearson's correlation analysis was performed to assess the relationship between PTK2 and ITGB1 mRNA expression levels using TCGA expression data across multiple cancer types. Scatter plots were generated and Pearson's correlation coefficients (r) were calculated. $P < 0.05$ was considered statistically significant.

Protein-protein interaction network and enrichment analysis

To understand the interactions and functional relationships of PTK2, the STRING database (https://stringdb.org/) was utilized. STRING integrates known and predicted protein-protein associations, including direct (physical) and indirect (functional) interactions. It incorporates multiple types of evidence including protein homology, text mining, genetic interactions, and shared pathway involvement. STRING was used to visualize the interaction network for PTK2 and related genes. Minimum required interaction score was set to medium confidence (0.400).

Statistical analysis

Survival outcomes are presented as hazard ratios accompanied by their p-values, obtained from the logrank test. Spearman's correlation was adopted for comparing PTK2 with other genes, which

also incorporated data from TIMER. The association between PTK2 expression and tumor-infiltrating immune cells was further refined by employing partial correlation analysis, eliminating the influence of tumor purity. Pearson's correlation analysis was executed to evaluate the relationships between PTK2 and other genes. A significance level was set at $P < 0.05$.

Result

Role of PTK2 in all cancers with a focus on bladder cancer

Our analysis indicates that elevated expression of the focal adhesion kinase (FAK) gene, also known as PTK2, may be associated with an increased risk of various malignancies. We examined mRNA expression data from the TIMER database and observed higher PTK2 transcript levels in tumor tissues compared to normal tissues (Figure 1). We next focused our analysis on bladder urothelial carcinoma (BLCA) by examining PTK2 mRNA expression data from 408 BLCA tumor samples and 19 normal tissue samples. A statistically significant increase in PTK2 mRNA expression was detected in BLCA tumors relative to normal tissues (p = 2.480200E-03, Figure 2A), suggesting PTK2 overexpression may be implicated in BLCA pathogenesis.

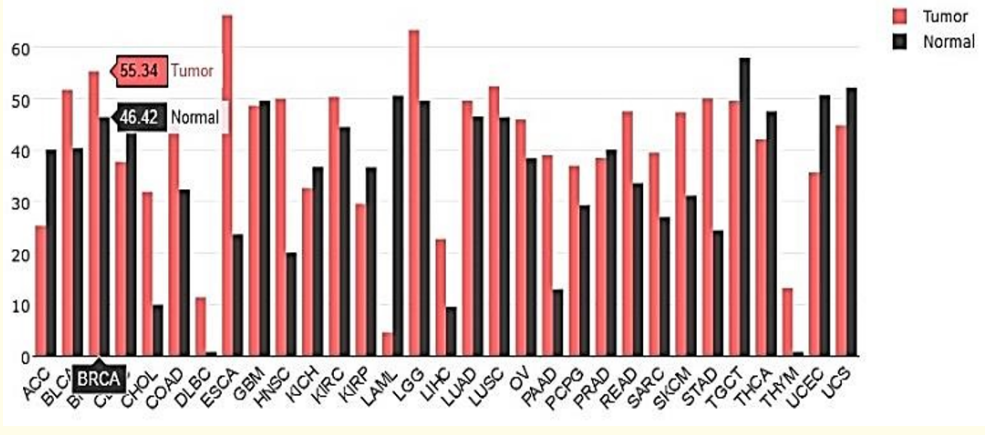

Figure 1: Higher expression of PTK2 was found in tumors as compared with non-tumor tissues in patients with bladder cancer.

To further evaluate PTK2 expression in BLCA, we performed subgroup analyses of PTK2 mRNA levels across different tumor stages and genders using data from the UALCAN database. However, PTK2 expression did not significantly differ between males and females (p = 7.118200E-01, Figure 2B), suggesting gender is not a major determinant of PTK2 expression in this malignancy. Our analysis revealed significantly higher PTK2 expression in stage 1, 2, 3, and 4 BLCA tumors relative to normal tissues (p = 2.640200E-02, 3.964600E-04, and 1.692210E-02, 4.500100E-02 respectively; Figure 2B). Furthermore, PTK2 expression was decreased in stage 3 and 4 tumors compared to stage 2 tumors (p = 4.716000E-02 and 3.338200E-02, respectively; Figure 2C), indicating PTK2 levels may correlate with BLCA progression.

PTK2 relation with other markers

To investigate the relationship between PTK2 and ITGB1 (Integrin beta-1) expression, we utilized the TIMER online tool [23]. TIMER allows for evaluation of miRNA targets, gene signatures, and immune infiltrates across tumor samples from The Cancer Genome Atlas (TCGA). We input PTK2 as the query gene and selected all available cancer studies, comprising over 10,000 samples across 32 cancer types. Spearman's correlation analysis was performed to assess the association between PTK2 and ITGB1 mRNA expression across this diverse set of tumors. A positive correlation was identified between PTK2 and ITGB1 expression in purity-adjusted analysis (r = 0.177 and p-value of 6.47e-04) (Figure 3A). Correlation analysis without purity-adjusting, also revealed that there is a positive correlation between these two genes expression (r = 0.079 and p = 1.1e01) (Figure 3B).

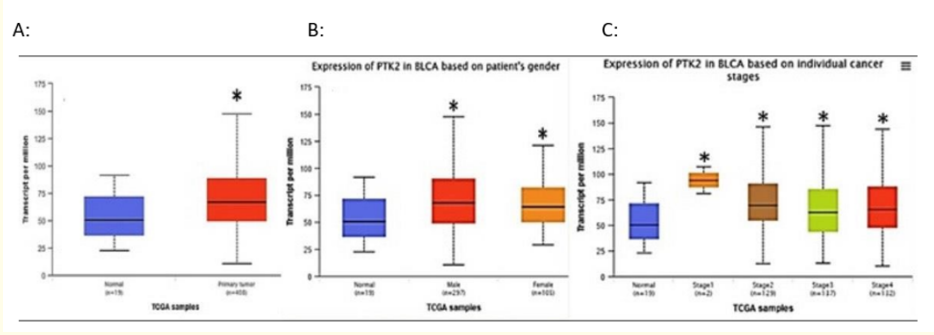

Figure 2: (A) PTK2 transcription was significantly higher in patients with BLCA as compared with normal subjects. (B) subgroup analysis showed no deference between male and female patients. (C) PTK2 expression based on individual cancer stages revealed a remarkably higher expression in stage 2 as compared with stages 3 and 4 using ULCAN database.

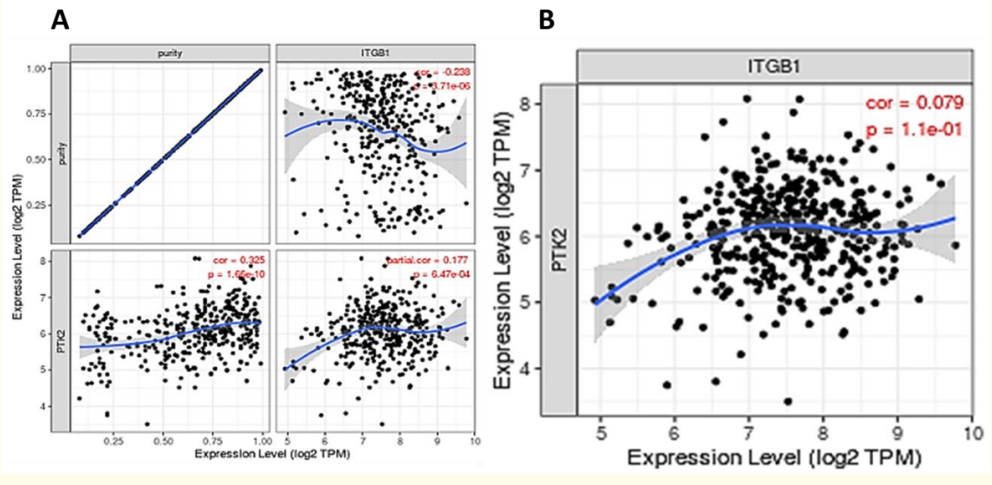

Figure 3: (A) Purity-adjusted correlation analysis between PTK2 and ITGB1 genes. (B) PTK2 expression is correlated with the expression of ITGB1 in BLCA.

We used the STRING online bioinformatics tool (version 12.0) to investigate the possible protein-protein interactions of PTK2. The tool allows us to create protein-protein interaction networks based on data from several biological databases, including MINT, HPRD, BioGRID, DIP, COG, and Reactome [24]. We input PTK2 as the query gene and selected Homo sapiens as the organism. We used the following parameters: search by name, confidence network edge, maximum interaction, and show physical and predicted interactions. The protein-protein interaction network that we obtained showed PTK2's interactions with several proteins, including GRB2, BCAR1, CRK, PIK3R1, ITGB1, ITGB3, VCL, SRC, PXN, and PTEN. Figure 4 presents a visualized form of this network, where nodes represent proteins and edges represent proteinprotein associations based on evidence from curated databases. The thickness of the edges correlates with the confidence score for that interaction, according to the weighting algorithm used by the STRING tool.

PTK2 and immune cell infiltration

In the present study, we utilized the TIMER database to investigate the correlation between PTK2 gene expression and immune cell infiltration in bladder cancer (BLCA) (Figure 4). Spearman's rank correlation analysis revealed that PTK2 expression exhibited a significant positive association with infiltration of B lymphocytes (r = 0.175, p = 7.96x10-4) and macrophages (r = 0.131, p =

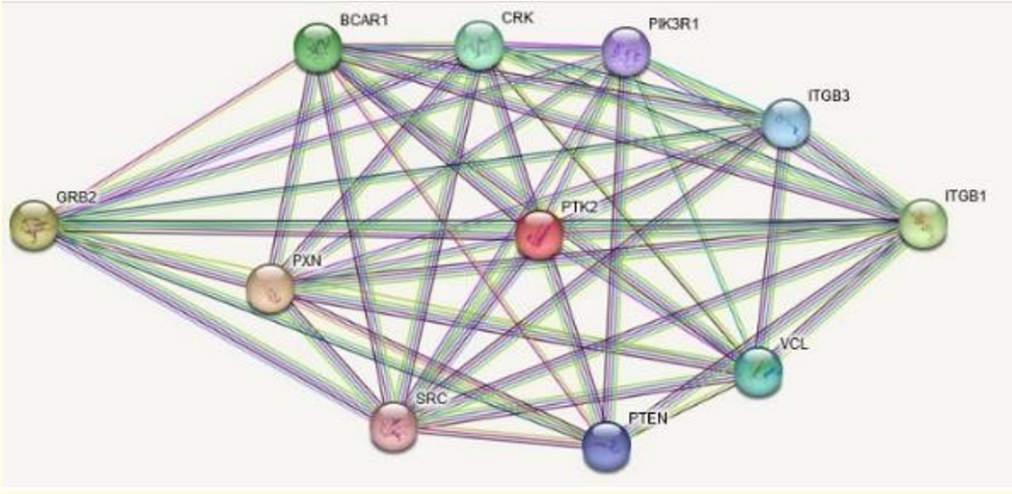

Figure 4: PTK2 interacting protein networks in the bladder cancer. We analyzed PTK2-coexpressed genes retrieved from the String (version 12). Clustered molecules are represented as nodes tagged with their gene symbols. The interacting strength between 2 nodes is represented by the thickness of the line. Minimum required interaction score was set to medium confidence (0.400).

1.20x10-2). In contrast, PTK2 expression was negatively correlated with infiltration of CD4+ T cells (r = -0.12, p = 2.18x10-2) and dendritic cells (r = -0.258, p = 5.63x10-7) in BLCA tumors. No significant correlations were observed between PTK2 and CD8+ T cell or neutrophil infiltration (p = 4.02x10-1 and p = 2.44x10-1, respectively). Furthermore, Kaplan-Meier survival analysis using the TIMER database demonstrated that increased CD8+ T cell infiltration was associated with improved prognosis in BLCA, with a statistically significant difference in overall survival between high and low CD8+ T cell infiltration groups (p = 0.007). Together, these analyses provide evidence for an association between PTK2 expression and the tumor immune microenvironment in BLCA as shown in Figure 5.

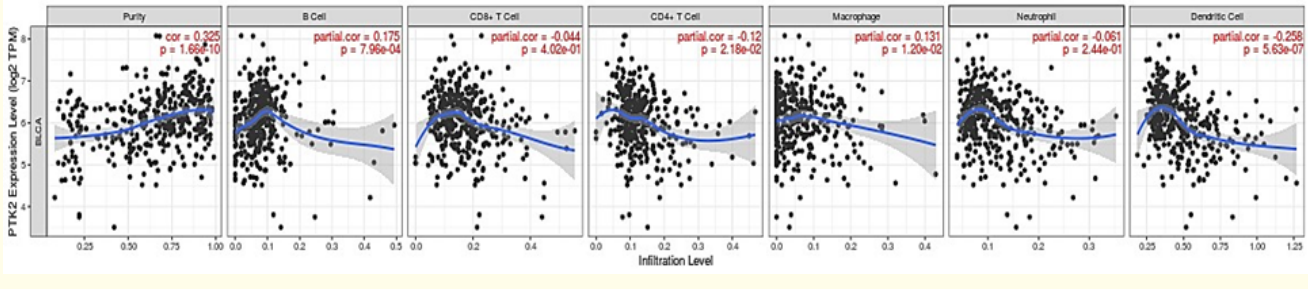

Figure 5: PTK2 expression is correlated with the level of immune cells (B Cell, CD8+ T Cells, CD4+ T Cells, Macrophage, Neutrophil, and Dendritic Cell) infiltration in BLCA.

Discussion

Bladder cancer is a common type of cancer that is often misdiagnosed or improperly treated and has a high recurrence rate, even in well-managed patients [25-27]. To address this issue, target therapies that can precisely kill cancer cells and minimize side effects are required for long-term disease management. Our study primarily focused on PTK2, known to play a pivotal role in bladder cancer progression [9,28,29]. Notably, our analysis revealed a significant surge in correlation between PTK2 mRNA expression and the advanced stages of bladder cancer, reinforcing its potential role as a therapeutic target.

PTK2 has been identified as a key player in the invasion, progression, and metastasis of a plethora of cancers, encompassing not just bladder cancer but also hepatocellular, thyroid, rectum, ovary, and several others [11,30,31]. The gene produces a cytoplasmic protein of FAK, which contributes to various signaling pathways of cancer growth and metastasis [32]. FAK has a fundamental role in tumorigenesis, providing sustained proliferative and survival signals by overriding anoikis, a form of apoptosis in the extracellular matrix, through both autophosphorylation and kinase activity [33,34]. Moreover, FAK is involved in migration, invasion, epithelial-mesenchymal transition, and angiogenesis, primarily focusing on metastasis and cancer progression stimulation [35].

Several experimental studies have investigated the impact of FAK on bladder cancer progression and invasion. The study by Kong et.al found that tyrosine phosphorylation of SRC is a key mediator of FAK related migratory and invasive activity, and the activation of FAK itself is a crucial factor in the transformation of growth factor beta-induced migration and invasion [36]. The FAK protein plays a critical role in the regulation of apoptosis and survival signaling in bladder cancer cells. Upon activation by specific extracellular stimuli, it interacts with phosphatidylinositol 3-kinase (PI3K), which in turn activates the PI3K/Akt pathway. This pathway leads to the phosphorylation of Akt, ultimately influencing the apoptosis and survival of bladder cancer cells [37]. Our previous research indicates a correlation between FAK mRNA expression over time and post-surgery recurrence. These findings suggest that the augmented expression of FAK mRNA could be attributed to the augmented stiffness of the extracellular matrix (ECM) postsurgery. Our findings suggest that the activation of FAK, in conjunction with other molecules such as integrin as a mechanoreceptor and CDC42, may contribute to the recurrence of bladder cancer [17,38]. These findings paved the way for a bioinformatics-based exploration of PTK2's influence on bladder cancer.

Furthermore, our PPI network analysis revealed other proteins involved in bladder cancer stimulation with PTK2, including GRB2, BCAR1, CRK, PIK3R1, ITGB1, ITGB3, VCL, SRC, PXN, and PTEN which can be used to define target, combinatorial and molecular therapies [39,40]. Our previous experimental study revealed a strong correlation between ITGB1 and Fak, as confirmed by the PPI network results presented in this article. Combination therapy represents a successful strategy for enhancing therapeutic outcomes in the context of bladder cancer. This approach involves the use of different drugs, each with distinct mechanisms of action and molecular targets, to enhance efficacy, increase response rates, reverse resistance, and reduce toxicity. By targeting the tumor's heterogeneity through distinct pathways and dynamics, combination therapy has been shown to improve response rates and therapeutic outcomes. Furthermore, the combination of immunotherapy and conventional agents overcomes the limitations of individual therapies, such as low response rates and potential resistance, to provide a more comprehensive and effective treatment approach [41-43].

Additionally, our research has uncovered significant findings regarding the expression of PTK2 in bladder cancer and its relationship with immune cell infiltration. Our observations showed a strong, positive correlation between PTK2 and B lymphocytes and macrophages while revealing a negative correlation with CD4+ T cells and dendritic cells. Interestingly, we also found that CD8+ T cell infiltration is associated with a better prognosis. Macrophages play a critical role in tumor angiogenesis and inflammatory reactions, which are often triggered by hypoxic tumor tissues or the production of monocyte chemotactic factors by these hypoxic tissues [44,45]. However, CD4+ T cells, also known as T helper cells, enhance the overall anti-tumor immune response by releasing cytokines like IL-2, IL-10, IFN-γ, and TNF-α, which activate and coordinate other immune cells, especially CD8+ T cells. CD8+ T cells can identify intracellular antigens presented by MHC class I molecules expressed by tumor cells and are considered a better population for targeting tumor cells due to their ability to mediate anti-tumor cytotoxicity [46-49]. It is widely believed that B lymphocytes induce apoptosis, inhibit tumor growth, and kill tumor cells through cytotoxicity. However, B regulatory cells (Bregs), a subcategory of B lymphocytes, produce regulatory cytokines such as IL-10 and TGF-β, which suppress immune responses and promote tumor progression. Furthermore, Bregs can transform resting CD4+ T cells into regulatory T cells (Tregs), which further support tumor growth and make B lymphocytes a double-edged sword [50,51]. In conclusion, our findings underscore the pivotal role of PTK2 in bladder cancer, especially its interaction with immune cell infiltration, which might be the underpinning mechanism to pave the way for innovative therapeutic immunotherapies targeting PTK2.

While this study provides important preliminary insights into PTK2's potential significance in bladder cancer, there are key limitations that necessitate further rigorous research. A primary limitation of this study is the lack of experimental validation about correlation of PTK2 with immune cell infiltration, as the findings are

based solely on computational analysis of public databases. The differentially expressed genes, protein interactions, and immune associations require confirmation using molecular biology techniques. Additionally, the retrospective, aggregated patient data may introduce biases and prevent detailed subtype analysis. Finally, extensive preclinical validation through drug screening and animal models is required to confirm PTK2 as a therapeutic target due to its correlation with immune cells in tumor tissues. Our computational perspectives set the stage for meticulous experimental follow-up to overcome the inherent limitations of database mining and realize any potential translational promise.

Ethics and Consent to Participate

The study was found to comply with the national norms and regulations for conducting medical research in Iran as well as the ethical principles.

Consent for Publication

Not Applicable

Availability of Data and Material

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Competing Interests

The authors do not have any financial or other relationships, which could be regarded as a conflict of interest.

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Author Contributions

Study concept and design: HG and SAN; Acquisition of data and Statistical Analysis: HG and MZ; Drafting of the manuscript: FS and SAN; Critical revision of the manuscript for important intellectual content: SAN, FS, HG, and MZ. All individuals listed as (co)-authors have met the authorship criteria, and nobody who qualifies for authorship is omitted from the list. The final manuscript was corrected and approved by all authors.

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Authors Information

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