Bioinformatic analysis of important miRNA and Gene-Network analysis of FAT-1 gene, a key regulator of head and neck cancer.

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ABSTRACT :
Introduction: Head and neck cancer is considered as the sixth worldwide leading cancer which are caused due to alcohol and tobacco use, radiation exposure, genetic disorders, etc. MicroRNA is small, single stranded RNA which contains 18-25 nucleotides plays a significant role in post-transcriptional regulation of genes. Commonly FAT-1 acts as tumor suppressor but abnormalities in this leads to development of tumors.
Aim : Analysis of important miRNA and Gene network analysis of FAT-1 gene , a key regulator of head and neck cancer.
Materials and methods: Prediction of microRNA targets for FAT - 1 gene in humans was carried out using Target-scan Human software program. miTG scores of ≥0.99 were identified using the Dianalab software program. Gene network analysis of FAT - 1 was carried out by STRING database online server program. Target miRNAs were predicted using miRDB online server program.
Results: Bioinformatic analysis identified dysregulated miRNAs and hub genes associated with the FAT-1 signaling network, revealing significant involvement in cell proliferation, apoptosis, epithelial–mesenchymal transition, and metastatic pathways in head and neck cancer. Gene-network analysis demonstrated that FAT-1 interacts with multiple oncogenic and tumor-suppressive pathways, suggesting its central regulatory role in tumor progression. Important gene interactions with a combined score of ≥0.99 were considered and listed. miRNA targets with a target score of more than 95 were considered for further analysis.
Conclusion: The study of bioinformatic analysis of important miRNAs helps in understanding the involvement of the FAT-1 gene in head and neck cancer, where FAT-1 may act as a potential biomarker and therapeutic target due to its association with cancer-related miRNA regulation and molecular signaling pathways. Integrated miRNA and gene-network profiling further provides valuable insight into the molecular mechanisms underlying head and neck carcinogenesis.

Keywords: radiation exposure, genetic disorders, post-transcriptional regulation, tumor suppressor, abnormalities.

INTRODUCTION :

Head and Neck Squamous Cell Carcinoma (HNSCC) is the sixth worldwide leading cancer(1). Head and neck cancers arise from different anatomical sites like cancers of the oral cavity, salivary glands, sinuses, nasal cavity, tonsils, pharynx, etc (2). HNSCC is an aggressive malignancy associated with poor prognosis and therapeutic resistance. Major risk factors include tobacco use, alcohol consumption, radiation exposure, genetic disorders and infection with high-risk strains of Human Papillomavirus Infection. The disease frequently exhibits local recurrence and lymph node metastasis, contributing to disease progression (3)(4). Drugs used in treatment of advanced Head and neck cancers are pembrolizumab, nivolumab and immune checkpoint inhibitors show benefit in few cases (5). Despite the use of multimodal treatment approaches such as surgery, radiotherapy, chemotherapy, and immunotherapy, disease recurrence and resistance to therapy continue to limit successful clinical outcomes (1).
In human transcription only 2% are coding RNAs which encode proteins and remaining 98% are noncoding RNAs. microRNAs (miRNAs), ribosomal RNAs (rRNAs) and long noncoding RNAs (lncRNAs) are included in the 98% noncoding RNAs (6). MicroRNA is small, single stranded RNA and 18-25 nucleotides long, which has an important role in regulating gene expression. miRNA is transcribed by RNA polymerase II & III which function as locks for messenger RNAs and prevent their translation into proteins (7). miRNAs are considered as an attractive tool and the most promising biomarkers in cancer progression. Hence miRNAs have a role in biological processes like oncogenesis (8). These endogenous expressing small non-coding RNA plays an important role in post-transcriptional regulation of genes (9).

These molecules play a most crucial role in metabolism, differentiation, proliferation, migration, apoptosis and cellular development (10).

The FAT-1 gene encodes an omega-3 desaturase that modulates inflammatory signaling pathways associated with carcinogenesis. Dysregulation of FAT-1–related molecular networks, including miRNA interactions and gene regulatory pathways, may contribute to the progression of head and neck cancer (11). Thus, bioinformatic analysis of FAT-1–associated miRNAs and gene networks may help clarify its role as a potential biomarker and therapeutic target in head and neck cancer. In addition to its enzymatic function, FAT1 (FAT atypical cadherin 1) encodes an adhesion-related protein involved in cell–cell communication and developmental processes (12). It is frequently altered in human cancers and exhibits context-dependent roles, acting either as a tumor suppressor or oncogenic regulator. As a tumor suppressor, FAT1 is implicated in controlling cell proliferation, maintaining cell adhesion, and regulating extracellular matrix organization. Abnormal FAT1 expression influences multiple signaling pathways involved in cell growth, migration, and invasion, thereby contributing to tumor development and poor clinical outcomes (13), (14). The aim of the study is to analyze the important miRNA and Gene network analysis of FAT-1 gene , a key regulator of head and neck cancer.

MATERIALS AND METHODS :

Gene Network Analysis of FAT1:

The network analysis of the FAT1 gene, which encodes a cadherin-like adhesion protein crucial for regulating cell polarity, migration, and signaling pathways linked to head and neck cancer progression, was performed using the STRING database (string-db.org). STRING is a widely recognized bioinformatics resource that compiles protein–protein interaction data from diverse sources, including experimental studies, curated repositories, computational predictions, and literature mining. By integrating these datasets, STRING enables the visualization of complex molecular interaction maps, helping researchers uncover functional relationships among genes and proteins and providing insights into their roles in disease biology.

For this study, the FAT1 gene symbol was queried in STRING with Homo sapiens specified as the organism to ensure human-specific relevance. The resulting output generated a detailed interaction network highlighting proteins that either directly associate with FAT1 or are functionally connected through biological pathways. Each interaction is assigned a combined confidence score, reflecting the strength of evidence supporting the association. To ensure analytical rigor, only interactions with a confidence score of ≥0.99 were retained for further examination. This stringent cutoff minimized false positives and emphasized highly reliable connections, thereby producing a refined FAT1-centered network. Such a high-confidence dataset provides a robust framework for interpreting FAT1's functional role and exploring its mechanistic involvement in tumor initiation and progression.

Target scan prediction of microRNA Targets for FAT-1:

The prediction of microRNA (miRNA) targets for the FAT1 gene, a key regulator implicated in head and neck cancer, was performed using the TargetScan Human database. This tool identifies potential miRNA–mRNA interactions based on conserved and non-conserved binding sites within the 3′ untranslated region (3′UTR), including 8mer, 7mer, and 6mer seed matches complementary to miRNA seed sequences (Lewis et al., 2005). Conserved miRNA families were prioritized to enhance the biological relevance of predicted regulatory interactions.

Integrated cancer genomics data from the Genomic Data Commons (GDC) data portal maintained by the National Cancer Institute (https://portal.gdc.cancer.gov), which incorporates datasets from The Cancer Genome Atlas (TCGA) and other large-scale cancer genomics projects, were utilized to contextualize FAT1-associated regulatory miRNA profiles within head and neck cancer.

High-confidence miRNA interactions, including hsa-miR-4762-5p (miTG score ≥ 0.99), were further validated using DIANA tools (DianaLab) to strengthen prediction reliability. Subsequently, gene–protein interaction and network analysis of FAT1 was performed using the STRING database, where interactions with a combined confidence score ≥ 0.99 were considered. This enabled the construction of a high-confidence protein–protein interaction network to elucidate the molecular role of FAT1 in head and neck cancer pathogenesis.

Identification of hsa-miR-4762-5p targets

The specific target identification of the conserved miRNA hsa-miR-4762-5p was performed using the miRDB online server (mirdb.org), a bioinformatics resource for miRNA target prediction and functional annotation. miRDB employs a machine learning–based algorithm trained on high-throughput sequencing data to predict miRNA–mRNA interactions with high accuracy by integrating sequence complementarity, structural accessibility, and other predictive features. For this analysis, hsa-miR-4762-5p was queried to retrieve its predicted mRNA targets relevant to FAT1-associated regulatory networks in head and neck cancer. A stringent cutoff score of ≥ 95 was applied to ensure high-confidence target selection, and only strongly supported interactions were considered for downstream functional interpretation and gene-network analysis.

RESULTS:

Figure 1: TargetScanHuman visualization of predicted hsa-miR-4762-5p interaction sites in FAT1

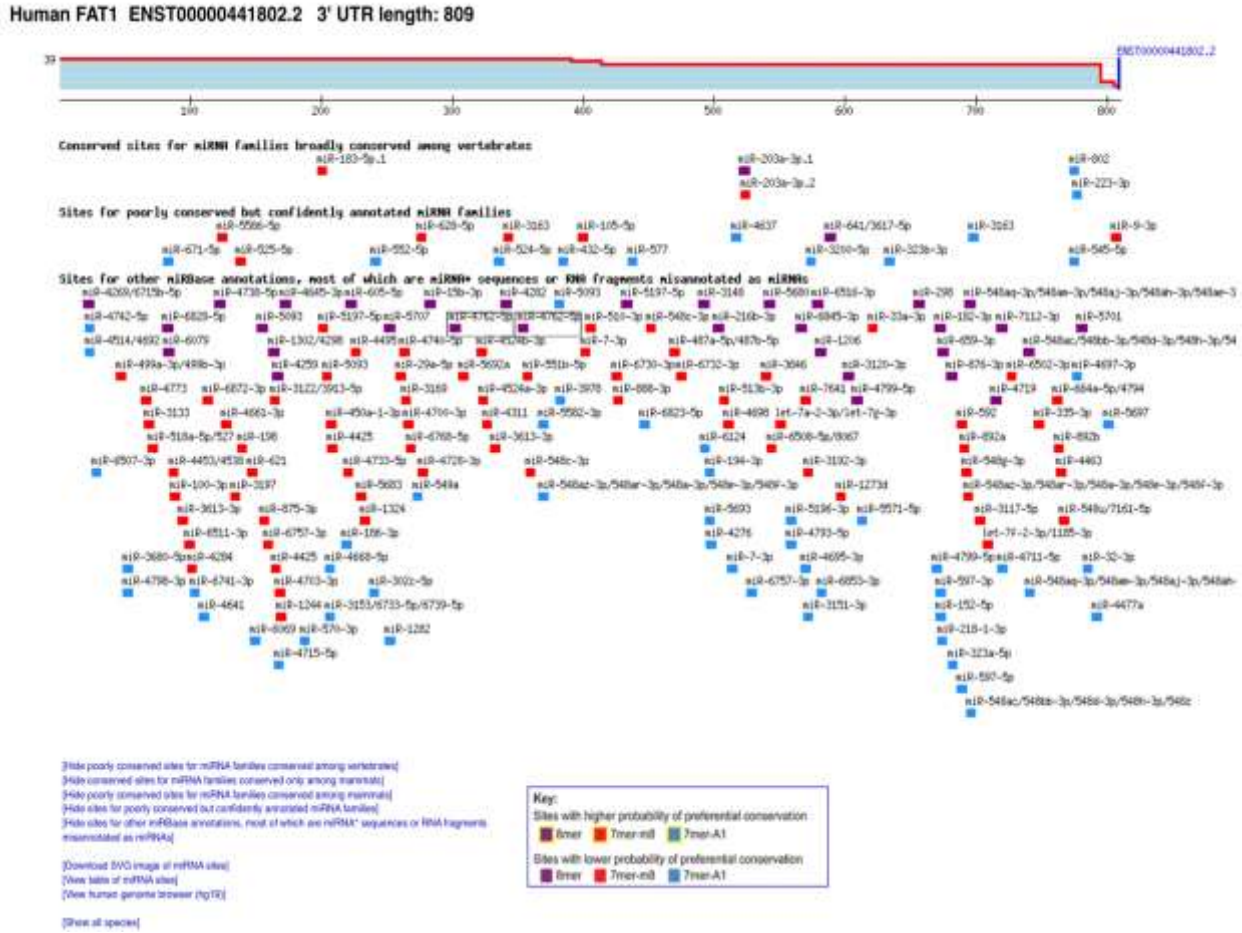


Figure 2: Cross-species conservation analysis of predicted hsa-miR-4762-5p binding regions within the FAT1 3′UTR

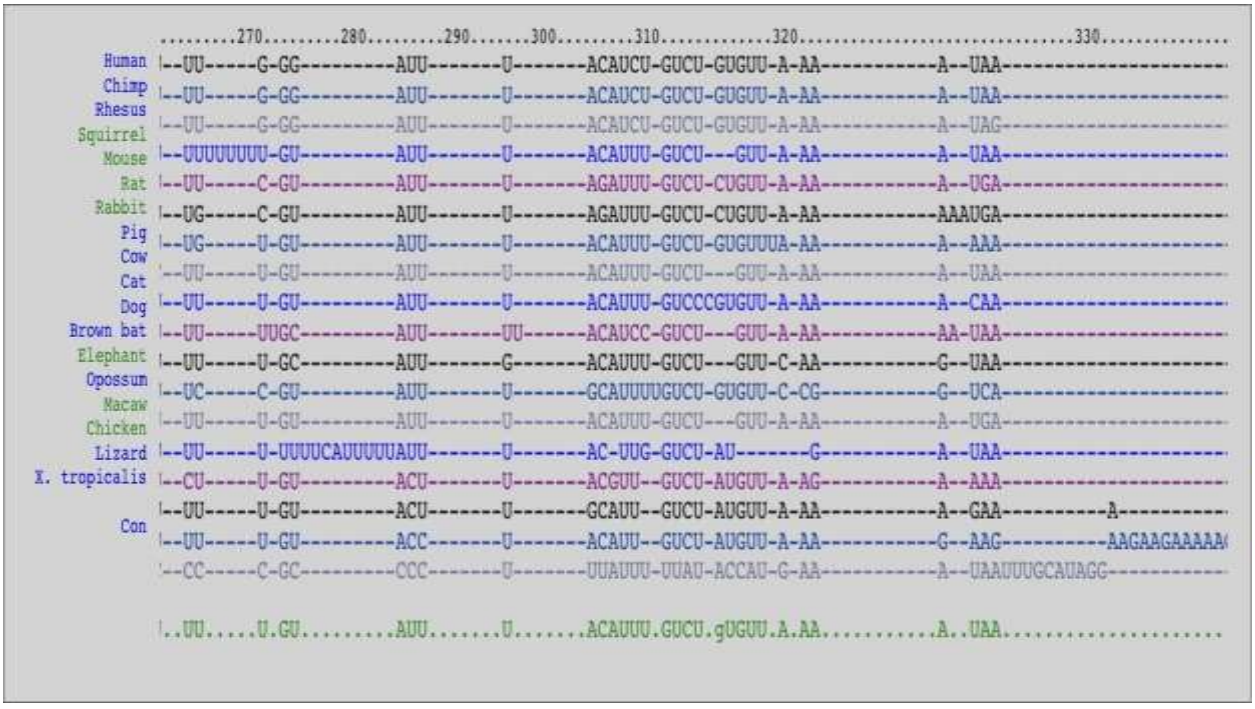
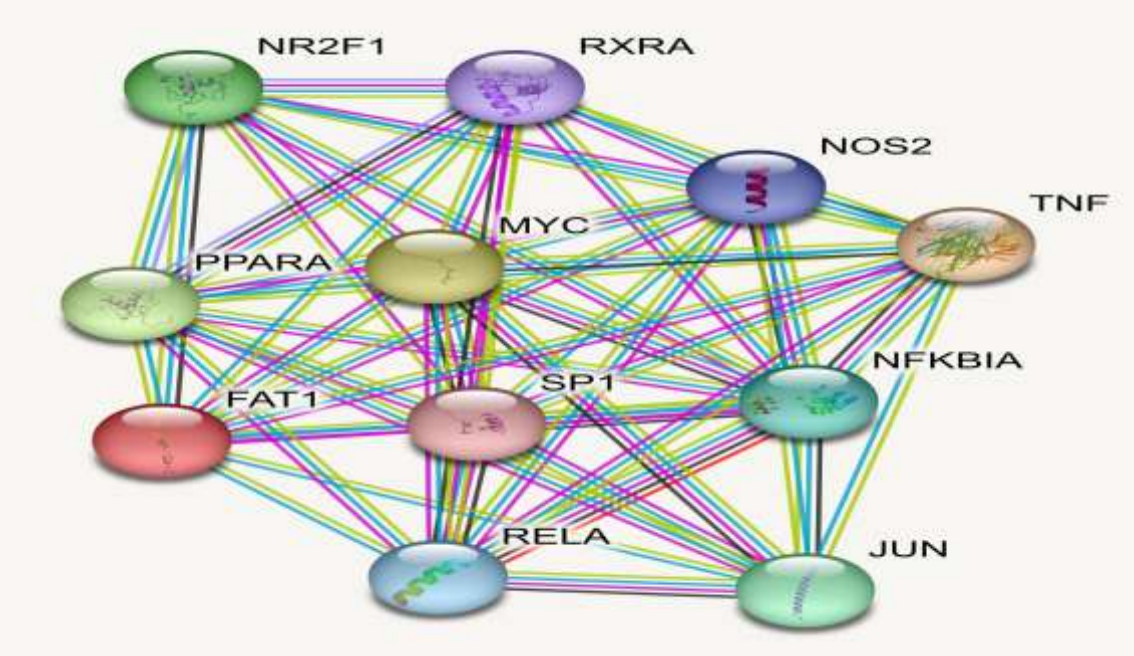


Figure 3: Prediction of miRNA for FAT1. These are few miRNAs related to Titin in which target scores of more than 95 were considered.

Target Rank	Target Score	miRNA Name	Gene Sequence	Important genes
1	100	hsa-miR-4762-5p	5' - ccaaaucuugaucagaagccu - 3'	UTY, RC3H1, PTBP3, VPS53
2	97	hsa-miR-7112-3p	5' - ugcaucacagccuuuggcccuag - 3'	ZDHHC17, ANO4, PPP6R2, SH3KBP1
3	97	hsa-miR-548x-3p	5' - uaaaaacugcaauuacuuuc - 3'	VGLL3, BDP1, GFPT1, CREBRF
4	97	hsa-miR-548aj-3p	5' - uaaaaacugcaauuacuuuua - 3'	VGLL3, BDP1, GFPT1, CREBRF
5	95	hsa-miR-548ae-3p	5' - caaaaacugcaauuacuuuca - 3'	PHC3, CREBRF, PTPRK, SOS2
6	95	hsa-miR-548aq-3p	5' - caaaaacugcaauuacuuuugc - 3'	PHC3, CREBRF, PTPRK, SOS2
7	95	hsa-miR-548ah-3p	5' - caaaaacugcaguuacuuuugc - 3'	CREBRF, PTPRK, B3GALT2, IKZF2
8	95	hsa-miR-548j-3p	5' - caaaaacugcauuacuuuugc - 3'	CREBRF, PTPRK, B3GALT2, IKZF2
9	95	hsa-miR-548am-3p	5' - caaaaacugcaguuacuuuugu - 3'	CREBRF, PTPRK, B3GALT2, IKZF2
10	95	hsa-miR-3613-3p	5' - acaaaaaaaaaagcccaacccuuc - 3'	EPHA7, SENP5, KMT2C, CSMD1

Figure 4: Represents the String diagram of FAT1 gene



To investigate the regulatory role of microRNAs in modulating FAT1 gene expression, predictive algorithms and protein–protein interaction analyses were employed.

Prediction of miRNA binding sites using TargetScanHuman identified hsa-miR-4762-5p as a high-confidence regulator of FAT1. Binding motifs across the 3′UTR of the FAT1 transcript (ENST00000441802.2) included multiple conserved site types (8mer, 7mer-m8, 7mer-A1), distributed along the 809-nucleotide region (Figure 1). The presence of preferentially conserved motifs suggests evolutionary pressure to maintain miRNA-mediated regulation of FAT1. Cross-species sequence alignment confirmed strong conservation of the hsa-miR-4762-5p binding region across mammals, with partial conservation in avian and amphibian lineages (Figure 2). This supports the functional relevance of the miRNA–FAT1 interaction in vertebrate biology.

High-confidence miRNA predictions (Target Score ≥95) yielded ten candidate regulators of FAT1 (Figure 3). Among these, hsa-miR-4762-5p achieved the highest score (100), while multiple members of the miR-548 family were recurrently identified. The associated target genes included regulators of transcription (CREBRF, PHC3), signaling pathways (PTPRK, SOS2), and chromatin modification (KMT2C), suggesting FAT1 is embedded within broader regulatory

networks. The STRING protein–protein interaction network contextualized FAT1 within cellular signaling pathways (Figure 4). FAT1 interacted with transcription factors (SP1, JUN, MYC, RELA), nuclear receptors (RXRA, NR2F1, PPARA), and inflammatory mediators (TNF, NOS2, NFKBIA). The clustering of transcriptional regulators and immune signaling molecules highlights FAT1's potential role in bridging cell adhesion and cytoskeletal organization with transcriptional control and inflammatory responses.

DISCUSSION:

This study provides evidence that FAT1 is a central regulator in head and neck squamous cell carcinoma (HNSCC), influenced by both microRNA interactions and protein–protein networks. Bioinformatic predictions identified hsa-miR-4762-5p and several members of the miR-548 family as strong regulators, with conserved binding motifs across vertebrates, suggesting evolutionary pressure to maintain miRNA-mediated control of FAT1. The identification of ten miRNAs with high target scores, linked to genes such as UTY, GFPT1, GAD1, CDH13, and CREBRF, highlights FAT1's integration into transcriptional, signaling, and chromatin-modifying pathways relevant to cancer progression. STRING analysis positioned FAT1 within clusters of transcription factors (MYC, JUN, SP1, RELA), nuclear receptors (PPARA, RXRA, NR2F1), and inflammatory mediators (TNF, NOS2, NFKBIA). This network suggests that FAT1 functions as a molecular hub, bridging cytoskeletal organization with transcriptional regulation and immune signaling. Such positioning explains its dual role: acting as a tumor suppressor in HNSCC while exhibiting oncogenic properties in oral squamous cell carcinoma (OSCC).

Experimental and clinical evidence supports this context-dependent behavior. Loss of FAT1 expression is associated with aggressive tumor features, recurrence, and poor survival, while knockdown studies demonstrate reduced proliferation, migration, and stemness with enhanced apoptosis (15). Conversely, FAT1 overexpression in OSCC promotes cell cycle progression, migration, and cisplatin resistance through deregulated signaling pathways. Clinical correlations with age, differentiation, and metastasis further emphasize its diagnostic relevance. Overall, FAT1 emerges as a multilayered regulator in head and neck cancers, controlled by miRNAs and protein networks, with implications for tumor progression, immune modulation, and therapy resistance (16). The integrative bioinformatic approach provides a systems-level perspective, supporting FAT1 as a promising biomarker and therapeutic target for precision-based strategies in HNSCC and OSCC (17).

Because of its tumor suppressing nature these surface receptors and signaling effects associated with FAT1 gene mutation are well identified remedial (or) therapeutic targets in Head and Neck Squamous Cell Carcinoma. Tumor downregulation is due to immune escape of tumor cells which is mediated by mutated FAT -1 gene Along with PD1 gene, leading to tumor immune tolerance (18). FAT1 functions as a tumor suppressor in head and neck squamous cell carcinoma (HNSCC). Mutations and reduced expression are closely associated with progression, recurrence, and poor survival. Clinically, FAT1 alterations correlate with lymphovascular invasion ($P < 0.001$) and variable protein expression across tumor tissues. Experimental evidence shows that exogenous FAT1 domains inhibit migration and invasion, indicating that mutation-driven loss enhances metastatic potential. Bioinformatic integration of miRNA prediction and protein–protein interaction networks provides a systems-level perspective on FAT1 regulation. These findings establish FAT1 as a candidate biomarker and therapeutic target in HNSCC (19).

FAT1 shows dual roles in oral and head and neck cancers, acting as both a tumor suppressor and oncogenic driver depending on context. Loss of FAT1 is linked to aggressive behavior, poor prognosis, recurrence, and therapy resistance, while knockdown studies demonstrate reduced proliferation, migration, and stemness with enhanced apoptosis. Conversely, FAT1 overexpression in OSCC promotes cell cycle progression, migration, and cisplatin resistance via deregulated signaling pathways. Clinical correlations with age, differentiation, and metastasis further suggest diagnostic relevance. Overall, FAT1 exerts context-dependent effects and represents a potential biomarker and therapeutic target in HNSCC and OSCC (20).

CONCLUSION :

This bioinformatic analysis demonstrates that FAT1 is regulated by specific microRNAs, notably hsa-miR-4762-5p, with conserved binding motifs supporting evolutionary significance. Gene-network mapping places FAT1 within transcriptional, signaling, and immune pathways, highlighting its role in tumor progression and therapy resistance. These findings establish FAT1 as a context-dependent regulator in head and neck cancer and suggest its potential as a biomarker and therapeutic target.

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CONFLICT OF INTEREST:

All the authors declared that there was no conflict of interest in the present study.

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