ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Hypermethylation of the GATA4 Gene in OSCC Patients in North Indian Population.

Aabhas Agarwal, Shivani Tyagi, Sakshi Chauhan, and Abhimanyu Kumar Jha*.

Department of Biotechnology, Sharda School of Engineering and Technology, Sharda University, Greater Noida-201310, India.

ABSTRACT

GATA4 (GATA binding protein 4) is a transcription factor encoding gene, pivotal in regulating gene expression by binding to specific DNA sequences, thereby orchestrating various cellular processes like cell differentiation, proliferation, and survival. Its interactions with other transcription factors, co-factors, and regulatory proteins allow for the modulation of gene expression patterns crucial for cancer development and progression. Genetic alterations leading to decreased GATA4 activity are observed, notably in oral cancer, predominantly emerging cancer attributed to genetic and epigenetic changes. Epigenetic modifications, which vary across populations due to dietary and environmental influences, contribute significantly to OSCC. Tobacco chewing and smoking are implicated as the key risk factors in OSCC pathogenesis. Notably, prior literature lacks reports on hypermethylation of the GATA4 gene. The findings obtained from this study would significantly contribute to understanding the potential of the GATA4 gene as a diagnostic biomarker.

Keywords: Cancer, Epigenetics, Hypermethylation, Tumor suppressor gene.

https://doi.org/10.33887/rjpbcs/2024.15.3.12

Corresponding author

INTRODUCTION

Cancer is a condition marked by unregulated cell growth throughout the body, making it among the most destructive types of human disease. It presents a wide range of distinct clinical features and leads to millions of deaths each year on a global scale. OSCC is a prevalent form of cancer impacting the oral cavity, encompassing areas such as the tongue, gums, and lips. Cancerous growth in the oral cavity can occur in various parts, including the lips, tongue, gums, mouth base, cheeks, and palate. The majority of cases, around 32%, are found in the inner lining of the cheek (buccal mucosa), followed by 22% in the tongue, 11% in the palate, and smaller percentages in other areas such as the lower lip, floor of the mouth, gums, vestibule, and alveolus [1]. It has been reported that oral cancer stood as the 16th most prevalent cancer worldwide, with approximately 389,846 new cases and 188,438 fatalities documented (GLOBOCAN, 2022). Data from the NPCR, supported by ICMR, indicates that Ahmedabad's urban male population exhibits the peak Age-Adjusted Rate (AAR) for oral cancer at 12.9, followed by Bhopal at 9.9. Among females, Assam's Kamrup district records the peak AAR at 6.5, with Bengaluru following closely at 5.8 [2].

Contrary to the developed world, where oral cancer is less prevalent, Nations in South Asia such as India, Sri Lanka, and Pakistan have higher incidences, attributed partly to betel nut consumption [3]. Meanwhile, Melanesia boasts the utmost global rates of incidence for oral cancer in both sexes (GLOBOCAN, 2022). Notably, oral cancer stands as the leading contributor to fatalities associated with cancer among men in India. Various risk factors, including alcohol consumption, smoking, HPV infection (linked to oropharyngeal cancers), and sun exposure (associated with lip cancer), contribute to elevated rates of incidence in Australia and New Zealand, as well as Eastern and Western Europe [4]. Epigenetics encompasses heritable alterations in gene expression that are independent of modifications to the DNA sequence itself. These changes are vital in regulating the cell cycle and differentiation, and they help prevent the mutation of genes [5]. It has a remarkable impact on regulating the expression of genes and the structure of chromatin by establishing a lasting mechanism for silencing genes [6]. The silencing of genes through promoter methylation is a critical factor in the development of cancer. Characteristics in the methylation of DNA hold potential for cancer diagnostics, serving purposes in both disease categorization and detection due to their occurrence at the early stages of cancer development [7]. When TSGs operate frequently, they restrain the tumor growth and abnormal functioning leads to Tumorigenesis. Mechanisms such as allelic deficiencies, genetic alterations, and hidden deletions are commonly implicated in the deactivation of these genes. TSGs can be categorized into the following types: those directly regulating the growth of tumors by impeding cellular replication and those whose deactivation induces instability of genes, subsequently fostering mutations that enhance Tumor growth [8].

GATA4 belongs to the family of zinc finger transcription factors, a group that encompasses 6 members ranging from GATA1 to GATA6. The structure comprises two distinctive transcription activation domains located at the N-terminus, accompanied by two central zinc finger domains. Following these features, it contains an NLS (nuclear-localizing signal) right after its second zinc finger, and a region located at the C-terminal. It significantly contributes to coordinating the expression of numerous target genes essential for organogenesis and responds dynamically to environmental signals. Serving as a pioneer modifier, it can effectively open closed chromatin structures, enabling the binding of itself and other transcription factors to specific target sites. Moreover, GATA4's function is intricately controlled through diverse post-translational alterations, such as phosphorylation, acetylation, methylation, and SUMOylation. This intricate regulation highlights the pivotal role of GATA4 in governing cellular fate determinations [9]. In oral squamous cell carcinoma (OSCC), GATA4 demonstrates varied roles, significantly influencing the aggressive nature of the condition. It functions as a stimulator of tumor growth, promoting the multiplication of cancer cells within the oral cavity. Moreover, GATA4 plays a crucial part in facilitating invasion, allowing cancer cells to penetrate adjacent tissues and disseminate throughout the oral area. Additionally, it aids in promoting metastasis, enabling the circulation of cancer cells from the primary site of the tumor to distant sites, thereby intensifying the advancement of the disease. Present study focuses on hypermethylation status of GATA4 gene in OSCC among North Indian Population as previous studies demonstrated that LATS1 and LATS2 genes are hypermethylated in OSCC among North Indian population [10], [11].

ISSN: 0975-8585

MATERIALS AND METHODS

Sample Collection

Between October 2023 to March 2024, Blood samples were obtained from OSCC-diagnosed patients with their informed consent, following ethical clearance from Dharamshila Cancer Hospital & Research Centre, New Delhi. In parallel, blood specimens were also collected from healthy individuals to act as a comparative control group.

DNA Extraction

DNA was isolated from blood samples and treated with a digestion buffer containing 10mMTris-HCl(ph-8), 10mM EDTA, 150mM NaCl, 10% SDS, and proteinase K. The DNA extraction was then carried out using the phenol-chloroform method followed by ethanol precipitation.

Sodium Bisulfite Modification

A total of 20µg of DNA extracted from the sample underwent sodium bisulfite treatment, a process designed to selectively convert all unmethylated cytosines to uracil while leaving methylated cytosines unaltered. This procedure was executed by utilizing a kit named EZ DNA Methylation-Gold Kit sourced from Zymo Research, headquartered in Orange, United States of America, following the manufacturer's outlined standard protocol.

MSP (Methylation-specific PCR)

Methylation-specific PCR (MSP) was conducted to anticipate the status of cytosine bases, offering precise resolution in detecting DNA methylation.

For the MSP reactions targeting GATA4-UM and GATA4-M, a 20µL reaction volume was prepared, comprising 2.5µL of 1× PCR buffer, 1µL of dNTP mixture, 0.5µL of each primer, 1µL of Taq DNA polymerase, and 2µL of bisulfite-modified DNA. The amplification procedure started with an initial denaturation stage at 95 °C for 10 minutes utilizing a thermocycler. Subsequently, cycling conditions included denaturation at 94 °C for 30 seconds, annealing at 60 °C for 30 seconds (for methylated samples) and 62 °C for 30 seconds (for unmethylated samples), followed by a final extension at 72 °C for 5 minutes (35 cycles). The resulting reaction samples were subsequently subjected to electrophoresis on a 2% agarose gel to observe the amplified products. The primer sequences utilized in the MSP reactions are detailed in **Table 1.**

Table 1: Primer sequence

Gene	Sequences (5′–3′)	Annealing temp (°C)	Product size
GATA4 (methylated)	F: GTATAGTTTCGTAGTTTGCGTTTAGC	60°C	136bp
	R: AACTCGCGACTCGAATCCCCG		
GATA4 (unmethylated)	F: TTTGTATAGTTTTGTAGTTTGTGTTTAGT	62°C	136bp
	R: CCCAACTCACAACTCAAATCCCCA		

M, Methylated; U, Unmethylated

RESULTS

Isolation of DNA

The DNA isolated from the blood samples was subjected to electrophoresis on a 1% agarose gel and then visualized using a gel documentation unit. This step was performed to evaluate the integrity and quality of the DNA samples (**Figure 1**).

ISSN: 0975-8585

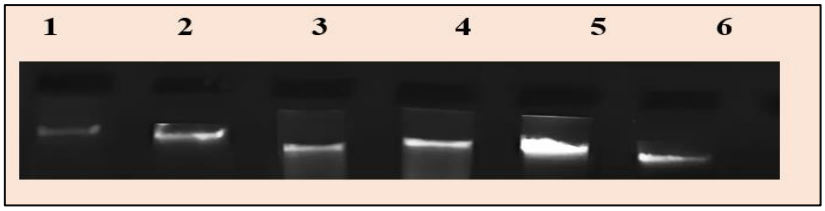

Figure 1: DNA extracted from blood samples obtained from OSCC patients and healthy control was analyzed. Lane No.1 to 4 represent DNA samples from OSCC patients, while Lane No. 5 and 6 represent DNA samples from healthy individuals (controls).

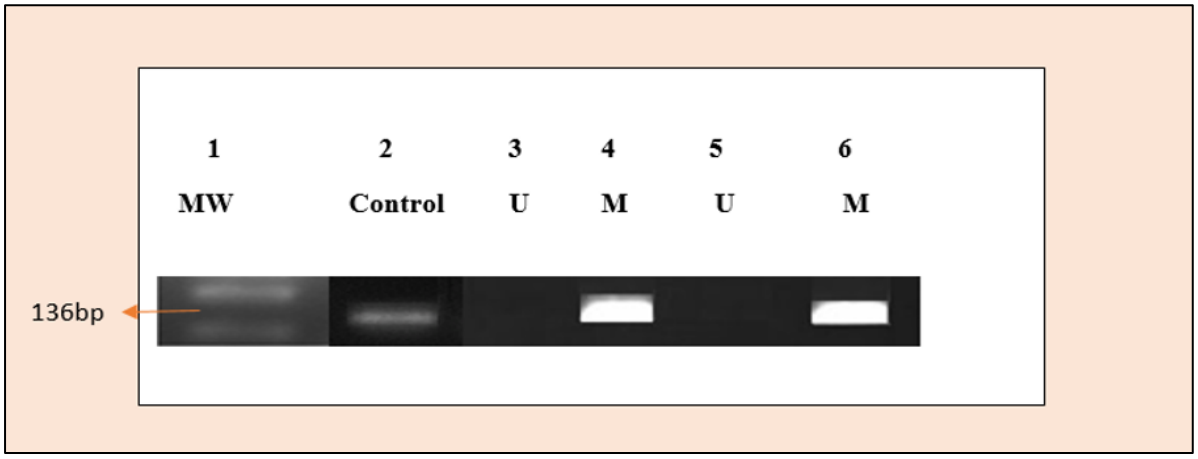

Figure 2: Lane No.1 represents molecular marker; Lane No.2 represents Positive control; Lane No.3 and 5 represent Unmethylated bands; Lane No.4 and 6 represent Methylated bands (136bp).

MS PCR Result of GATA4 Gene

Methylation-specific PCR (MSP) was utilized to examine the hypermethylation status of the GATA4 gene in North Indian OSCC patients (**Figure 2**).

Status of Hypermethylation

In 30% of OSCC patients, hypermethylation of GATA4 was detected. Among the 20 samples analyzed using Methylation-Specific PCR (MSP), 6 were found to be hypermethylated in OSCC patients. This study marks the first report from our research team on GATA4 hypermethylation in the North Indian population.

DISCUSSION

The findings from our study give valuable insights into the role of GATA4 in OSCC within the population of North India. Our results reveal a significant hypermethylation of the GATA4 gene in individuals diagnosed with OSCC in this region, suggesting a potential link between aberrant DNA methylation of GATA4 and the pathogenesis of OSCC among North Indian patients.

Li et al. (2020) demonstrated the pivotal role of GATA4 in promoting tumor growth, facilitating invasion, and enhancing metastasis in OSCC, corroborating our findings. Their study underscores the significance of GATA4 hypermethylation as a potential mechanism driving OSCC aggressiveness. The findings obtained from our study significantly shed light on the hypermethylation status of the GATA4 gene in OSCC among North Indian population and could be used as a potential diagnostic biomarker in future.

However, it is essential to acknowledge the regional variations in GATA4 methylation patterns and their association with OSCC. While our study focused on the North Indian population, future research should explore GATA4 methylation in other populations, including South Indian populations, to provide a comprehensive understanding of its role in OSCC.

CONCLUSION

In conclusion, our study underscores the significance of GATA4 hypermethylation in OSCC among North Indian patients. Further exploration of GATA4 methylation patterns in different populations, will enhance our understanding of OSCC pathogenesis and may result in the development of targeted therapeutic approaches.

ACKNOWLEDGMENT

The authors acknowledge the help provided by the Department of Biotechnology, Sharda School of Engineering and Technology, Sharda University, Greater Noida, India.

Abbreviations

- OSCC- Oral squamous cell carcinoma
- NPCR- National Program of Cancer Registries
- ICMR- Indian Council of Medical Research
- TSG- Tumor suppressor genes
- AAR- Age-Adjusted Rate
- MSP- Methylation Specific PCR

REFERENCES

[1] Kumar M, Jha AK. Exploring the potential of dietary factors and plant extracts as chemopreventive agents in oral squamous cell carcinoma treatment. Frontiers in Oral Health 2023;4.
[2] Dodda Junjannagari J. Microscopically Confirmed Pharyngeal Cancer in South India: An epidemiological case-control study. Acta Universitatis Tamperensis 2271 Tampere University Press Tampere 2017
[3] Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal For Clinicians 2021;71(3):209-49.
[4] Miranda-Filho A, Bray F. Global patterns and trends in cancers of the lip, tongue and mouth. Oral Oncology 2020; 102:104551.
[5] Jha AK, Sharma V, Nikbakht M, Jain V, Sehgal A, Capalash N, Kaur J. A comparative analysis of methylation status of tumor suppressor genes in paired biopsy and serum samples from cervical cancer patients among North Indian population. Russian Journal of Genetics 2016;52:226-30.
[6] Mahmood N, Rabbani SA. DNA methylation readers and cancer: mechanistic and therapeutic applications. Frontiers In Oncology 2019;9:489.
[7] Ehrlich M. DNA hypermethylation in disease: mechanisms and clinical relevance. Epigenetics 2019;14(12):1141-63.
[8] Hisaoka M, Tanaka A, Hashimoto H. Molecular alterations of h-warts/LATS1 tumor suppressor in human soft tissue sarcoma. Laboratory investigation 2002;82(10):1427-35.
[9] Gao L, Hu Y, Tian Y, Fan Z, Wang K, Li H, Zhou Q, Zeng G, Hu X, Yu L, Zhou S. Lung cancer deficient in the tumor suppressor GATA4 is sensitive to TGFBR1 inhibition. Nature Communications 2019;10(1):1665.
[10] Goel H, Mathur R, Syeda S, Shrivastava A, Jha AK. Promoter hypermethylation of LATS1 gene in oral squamous cell carcinoma (OSCC) among North Indian population. Asian Pacific Journal of Cancer Prevention: APJCP. 2021;22(3):977.
[11] Goel H, Singhal S, Mathur R, Syeda S, Gupta RK, Kumar A, Shrivastava A, Jha AK. Promoter hypermethylation of LATS2 gene in oral squamous cell carcinoma (OSCC) among North Indian population. Asian Pacific Journal of Cancer Prevention: APJCP. 2020;21(5):1283.