BRIEF REPORT

Real-World Evidence of EGFR Targeted Therapy in NSCLC– A Brief Report of Decade Long Single Center Experience

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ABSTRACT

The significance of EGFR targeted therapy in the lung adenocarcinoma is paramount. Several controlled clinical trials have reported considerable survival of EGFR mutation positive patients on receiving the EGFR tyrosine kinase inhibitor (TKI). However, the real-world evidence of benefits of EGFR TKI would be further useful to understand how the designated therapeutic regimen benefits the patients. In this study, we report a decade long real-world evidence of EGFR molecular testing in lung cancer at Tata Memorial Hospital (Mumbai, India). Laboratory and hospital records containing basic demographic details, clinical characteristics, treatment regimen, survival outcome were collected retrospectively. Statistical association and survival analysis were performed using the R programming. The cohort includes 9,053 lung cancer patients tested for EGFR mutations during 2011 to 2019. Baseline T790M and compound mutations were the only mutations observed co-occurring while all other EGFR mutations were mutually exclusive. Furthermore, the baseline T790M were also observed to be associated with TTF1 positivity, smoking and local metastasis. Overall survival of the patients harboring co-occurring compound mutations was significantly lesser than the other EGFR positive patients. Overall, our study suggests that EGFR TKI may provide real-world benefit to the lung cancer patients harboring mutually exclusive EGFR mutations. On the other hand, further systematic study is essential to develop better therapeutic regimen for co-occurring baseline EGFR T790M and other compound mutations.

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Drs. Prabhash and Chougule contributed equally to this work.

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Introduction

EGFR is driver oncogene providing oncogenic addiction, and therapeutic opportunity in lung cancers, most prominently in adenocarcinomas.[1–3] Most of oncogenic EGFR mutations in lung adenocarcinomas are observed in tyrosine kinase domain (exon 18–21), forming a mutational hotspot. First generation (e.g., erlotinib, gefitinib), second generation (e.g., afatinib) and third generation (e.g., osimertinib) tyrosine kinase inhibitor (TKI) targeting mutational hotspot reveal excellent therapeutic response and are standard of care in lung cancers.[4] Previously, we reported that EGFR hotpots mutations exist at intermediate frequency of approximately 25% in lung cancer patients of Indian origin as opposed to approximately 15% in western countries and approximately 60% in east Asian countries.[5,6] We also reported the efficacy of EGFR TKIs in lung cancers as a monotherapy[7,8] and in combination.[9] However, the laboratory studies and controlled trials often limit our interpretation to conditioned data. Here we report a decade long real-world EGFR clinical testing data from Tata Memorial Centre (Mumbai, India), enabling us to capture relatively rare but noticeable occurrences of EGFR compound mutations, baseline T790M mutations, regional heterogeneity, and poor implication on patient survival.

Materials and Methods

Data Collection

We retrospectively collected lung cancer patient data on the basis of EGFR mutation test reports from laboratory/institute records at Tata Memorial Centre (Mumbai, India). The study was conducted following to the ethical principles guideline of Declaration of Helsinki ICH-GCP. All the patients which were tested in the laboratory during January 1, 2011 to December 31, 2019 were included. The sample used for EGFR mutation analysis was either a tissue block (if biopsy specimen was used) or fluid cell block (pleural or pericardial fluid).A nested-PCR method with in-house primer (TaqMan) probes was used to test EGFR hotspot mutations, as reported previously by our group.[5–7] The data was anonymized and deidentified before analysis.

Statistical Analysis

We used Microsoft office excel for primary data collection and storage and R programming language for statistical analysis. All patients positive for multiple EGFR mutations (exon 18 G719X, exon 19 deletions, exon 20 T790M, exon 20 insertions, and exon 21 L858R) were classified as EGFR compound mutations. Patient's clinical and demographic profile (age, sex, smoking history, metastasis, performance status, and tumor pathology) was noted from the lung cancer audit database. The landscape of mutation was visualized using the cBioPortal.[10] Mutual exclusivity and co-occurrence of mutations were tested using the odds ration which indicates the likelihood of two genes being mutually exclusive or co-occurring across the selected cases. The statistical significance of mutual exclusivity was assessed using Fisher's exact test (p-value) and Benjamini–Hochberg false discovery rate (q-value), as implemented in cBioPortal. Overall survival was measured from the date of registration to the day of death or date of last follow-up. The survival probabilities were computed using Kaplan-Meier method and compared among the groups using log-rank tests implemented in survival 3.2, and survminer 0.4.9 packages in R 4.0.2 programming.

Results

Demographic, EGFR Mutation Exclusivity, and Co-Occurrence

Over the period of 2011 to 2019 a total of 9053 lung cancer patients were tested for EGFR mutations. We found 2129 patients harboring EGFR mutations with the overall 24.9% mutation frequency in lung cancer (Supplementary Table 1). Our cohort of lung cancer includes histopathological classes of 6812 adenocarcinomas, 1086 Squamous cell carcinomas, 53 small cell carcinomas, and 14 large cell carcinomas. We observed EGFR mutation frequency 27.7% in adenocarcinoma, 6.7% in squamous, 5.7% in SCLC, and 7.1% in large cell carcinomas (Supplementary Tables 1 and 2).

EGFR mutations were most frequently observed as exon 19 deletions with overall frequency of 14% followed by substitution mutation in exon 21 (L858R) at 9% frequency, exon 20 insertions at 0.7% frequency, exon 18 (G719X) mutations at 0.5% frequency and exon 20 (T790M) mutations at 0.8% frequency (Fig. 1).The two most common mutations (exon 19 deletion and L858R, log2 odd < -3, $p < 0.001$, q < 0.001)were observed to be mutually exclusive while rest of the mutations were observed to be co-occurring with either of the common mutation (Fig. 1*A* and Supplementary Table 3). Interestingly, we observed baseline T790M mutations mostly as compound mutation co-occurring with L858R or exon 19 deletions ($p < 0.001$).

We observed EGFR mutation frequency to be heterogenous across various states of the country. The elevated frequency of EGFR mutation was observed

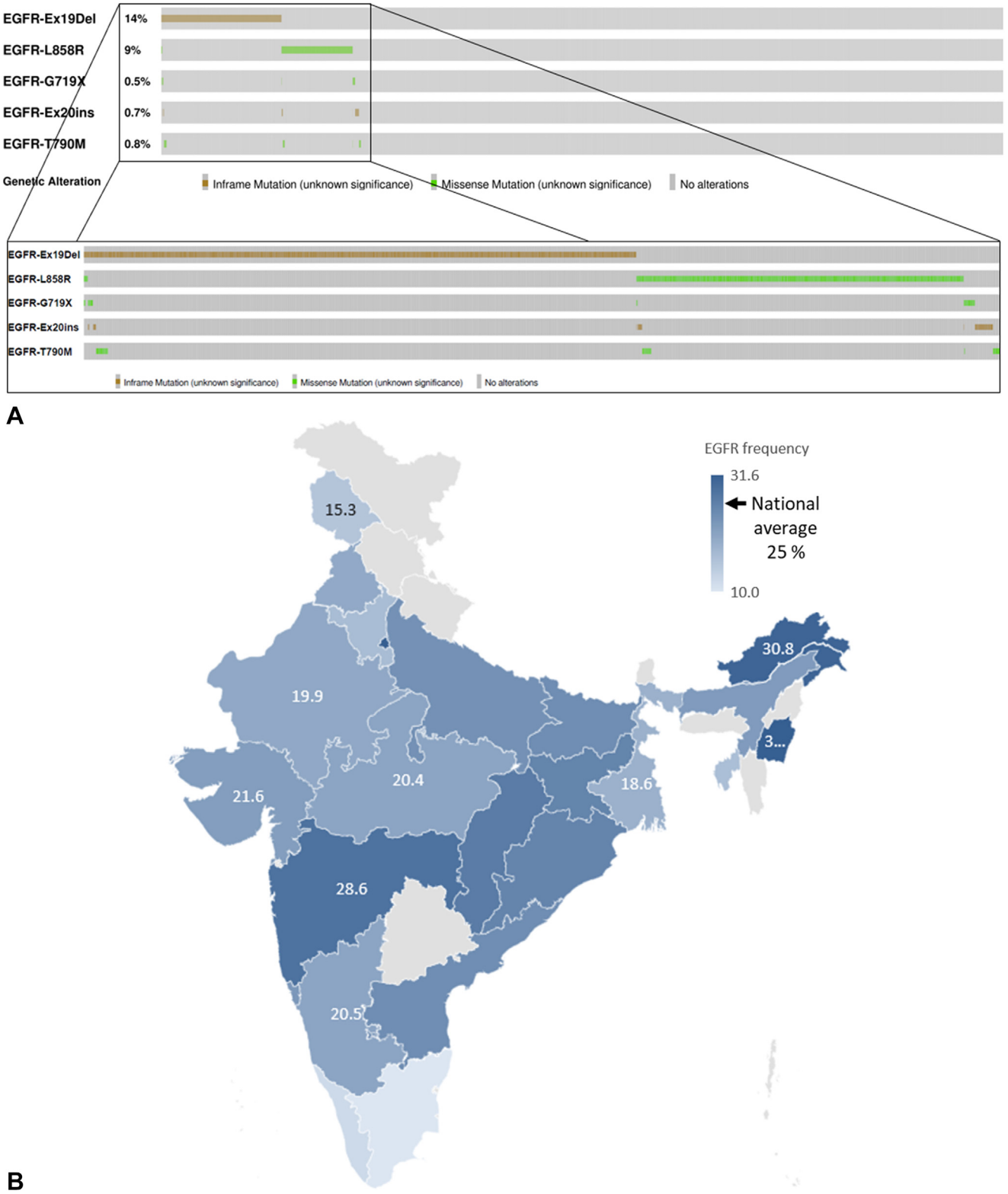

Figure 1. (*A*) Mutation heatmap depicting EGFR exon 19 deletion (14%), L858R (9%), G719X (0.5%), exon 20 insertion (0.7%) and T790M (0.8%) mutations as detected in lung cancer patients (N = 9053). The mutation heatmap reveals rare but important co-occurrence of various EGFR mutations (n = 92, 1.01%). (*B*) State-wise EGFR mutation frequency as observed at Tata Memorial Centre (Mumbai) from 2011 to 2019. The national average (24.9%) is shown with arrow on the color scale bar representing the complete range of 10% to 31.6% of EGFR mutation in lung cancer (N = 9053). Middle corridor of India including the NER states reveal elevated EGFR mutation frequency compared with other states. NER, North-East region.

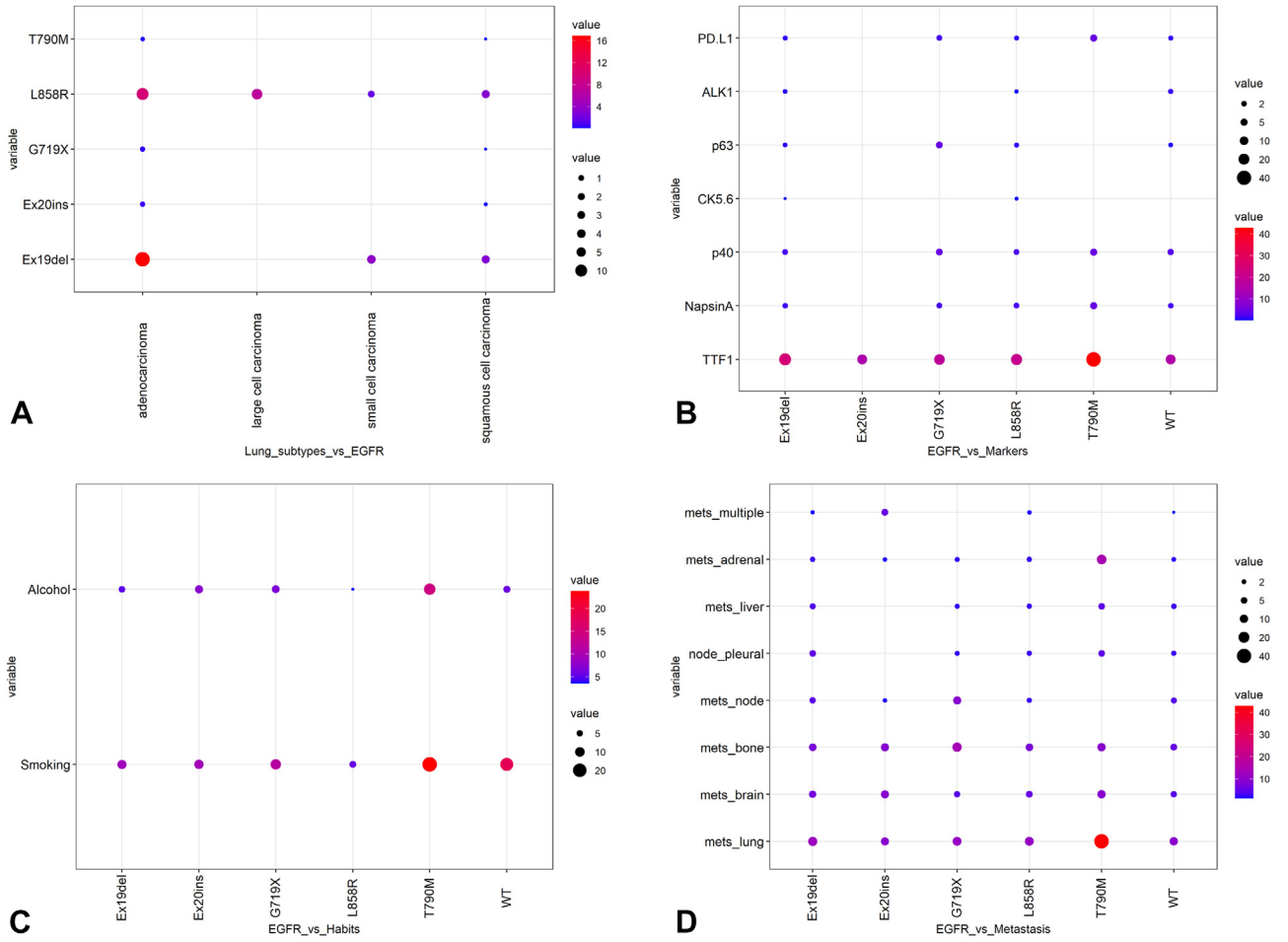

Figure 2. Mutation frequency map depicting association of various EGFR mutations with (*A*) histology, (*B*) histopathologic markers, (*C*) smoking habits, and (*D*) metastatic sites.

across the middle corridor of the country in contrast of the North and South region (Fig. 1*B*). Considering only the states having at-least 100 total cases evaluated (n = 9), we observed statistically significant difference in frequency of alterations across states (p = 2.98E-11; Supplementary Table 4).

EGFR Mutation Association With Sex, Smoking, Histopathologic Biomarker, and Metastatic Sites

The EGFR mutation frequency in male (19.87%) and female (31.97%) were found to be statistically significant (p= < 2.2e-16, Supplementary Tables 1 and 5). EGFR mutations were most frequently observed in adenocarcinoma (28.2%), followed by large cell carcinoma (7.14%), squamous cell carcinoma (6.72%), and small cell carcinoma (5.66%). Interestingly, baseline T790M mutations were observed at elevated frequency in patients with smoking and alcohol habit, although statistically non-significant (Fig. 2*A–D*). The baseline T790M mutations were also observed to be associated with locally advanced or adrenal gland metastasis (p = 0.002 and 0.005, respectively), and TTF1 positive tumors compared with others (p = 0.001; Fig. 2*A–D* and Supplementary Table 5).

Heterogeneity of EGFR Mutation Frequency in Different Regions of India

We observed EGFR mutation frequency to be heterogenous across various states of the country. The elevated frequency of EGFR mutation was observed across the middle corridor of the country in contrast of the North and South region (Fig. 3*A* and *B*). Considering only the states having at-least 100 total cases evaluated (n = 9), we observed statistically significant difference in frequency of alterations across states (p = 2.98E-11; Supplementary Table 4).

Compound EGFR Mutations Worsen the Patient Survival

Total 92 patients were observed to be harboring more than one EGFR mutations, hereafter called compound mutations. These patients revealed relatively

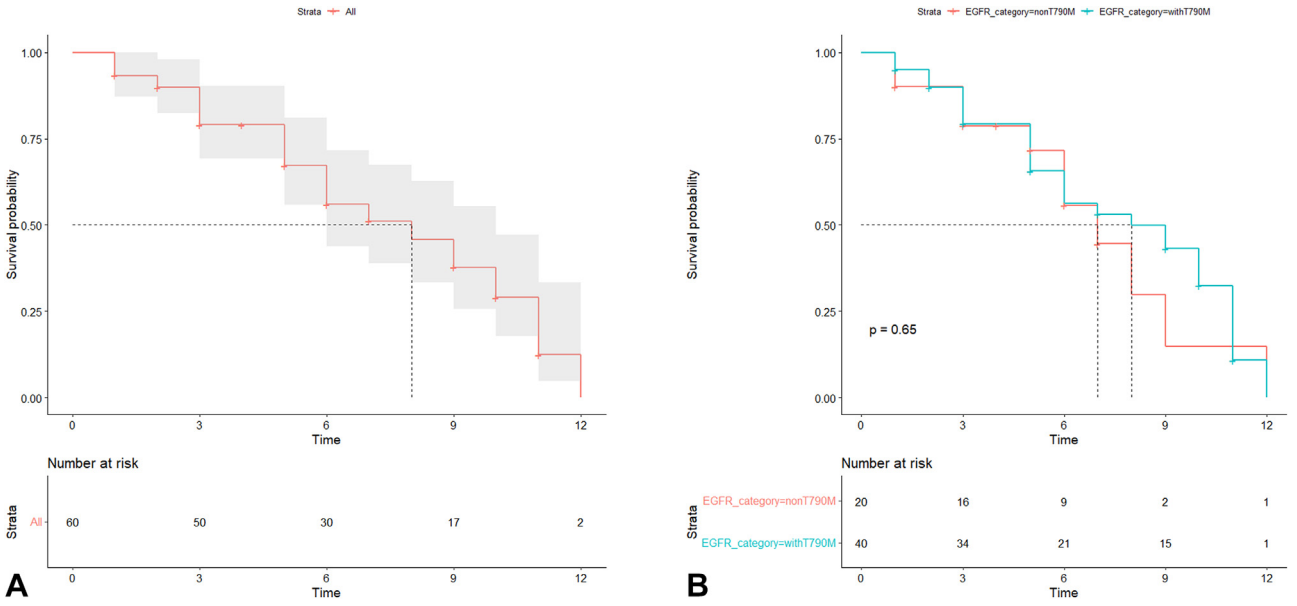

Figure 3. Overall survival analysis of EGFR compound mutation in response to EGFR TKI. (*A*) Kaplan-Meier curve of all EGFR compound mutation patients is shown (N = 92) with dotted line indicating median and gray area indicating 95% CI. (*B*) Kaplan-Meier curve of EGFR compound mutations with or without T790M mutations are shown. Dotted line indicates the median survival probabilities and the table below the plot shows number of patients at risk over various time points. CI, confidence interval; TKI, tyrosine kinase inhibitor.

poor overall survival (Fig. 3*A* and *B*; median = 8 mo, 95% confidence interval [CI]: 6–10 mo) compared with 21 months median survival of simple EGFR mutation harboring patients receiving first or second generation TKI, as we reported earlier.[7,11] Furthermore, the patients harboring baseline T790M as a part of compound mutations (n = 40) shows median survival probability of 8 months (95% CI: 6–11), like T790M negative compound mutation (median = 7 mo, 95% CI: 6–not applicable). On an average, 25% patients harboring compound mutation revealed favorable response to first line of therapy while remaining 75% patients revealed progressive disease (Supplementary Tables 6 and 7).

Discussion and Conclusion

We present one of the largest EGFR clinical genotyping datasets (N = 9053) of lung cancer in India. Our real-world data suggests statistically significant co-occurrence of baseline T790M with exon 19 deletion and L858R mutations. Our data is consistent with previous reports suggesting existence of rare baseline T790M mutations in lung cancer with implication in poor response to first generation TKI.[12,13] Furthermore, patients harboring non-T790M compound mutations reveal poor overall survival as well, indicating an overall reduced benefits to such patients irrespective of T790M status. Notably, we also observed baseline T790M mutation harboring patients in association with smoking and alcohol habit, TTF1 positivity, locally advanced, and adrenal metastasis. These observations have been possible owing to overall larger patient cohort, which was mostly a limitation in previous reports.[12,14,15] We further report that heterogeneity of EGFR mutation exists in various geographic region of the country, in line with previous reports of global inequality.[16,17] Given that population genetic diversity of India consists of more than 20 distinct genetic subpopulations and their admixture,[18,19] it is implication on cancer driver mutation is an open question. In conclusion, *EGFR* oncogene continues to lead perplexing questions highlighting the elevated compound mutation rate and association with population genetic diversity in India.

CRediT Authorship Contribution Statement

Anuradha Chougule: Conceptualization, Formal analysis, Investigation; Methodology; Project administration; Resources; Writing - review & editing.

Vanita Noronha: Data curation; Investigation; Methodology; Validation; Writing - review & editing.

Priyanka Pange: Data curation; Investigation; Methodology; Validation; Writing - review & editing.

Shrutikaa Kale: Data curation; Investigation; Methodology; Validation; Writing - review & editing.

Ankita Nikam: Data curation; Investigation; Methodology; Validation; Writing - review & editing.

Kavya Nambiar: Data curation; Investigation; Methodology; Validation; Writing - review & editing.

Dipika Marchande: Data curation; Investigation; Methodology; Validation; Writing - review & editing.

Arpana Durve: Data curation; Investigation; Methodology; Validation; Writing - review & editing.

Vinod Gupta: Data curation; Investigation; Methodology; Validation; Writing - review & editing.

Vinita Jagtap: Data curation; Investigation; Methodology; Validation; Writing - review & editing.

Priyanka Tiwrekar: Data curation; Investigation; Methodology; Validation; Writing - review & editing.

Nandini Menon: Data curation; Investigation; Methodology; Validation; Writing - review & editing.

Amit Joshi: Data curation; Investigation; Methodology; Validation; Writing - review & editing.

Rajeev Kaushal: Data curation; Investigation; Methodology; Validation; Writing - review & editing.

Trupti Pai: Data curation; Investigation; Methodology; Validation; Writing - review & editing.

Vijay Maruti Patil: Data curation; Investigation; Methodology; Validation; Writing - review & editing.

Amit Dutt: Writing - review & editing.

Shripad Dinanath Banavali: Writing - review & editing.

Pratik Chandrani: Conceptualization, Formal analysis, Investigation; Methodology; Project administration; Resources; Writing - review & editing.

Kumar Prabhash: Conceptualization, Formal analysis, Investigation; Methodology; Project administration; Resources; Writing - review & editing.

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Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *JTO Clinical and Research Reports* at www.jtocrr.org and at https://doi.org/10.1016/j.jtocrr.2023.100566.

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