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Original Research Article

Quantitative Analysis of Acute Phase Proteins in Chemo-Radiation–Induced Mucositis in Patients with Head and Neck Cancers

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Abstract: ***Background***: Head and neck squamous cell carcinoma (HNSCC) constitutes a major oncologic burden in India, with high incidence and mortality driven largely by tobacco-related disease.[1–4] Concurrent chemo-radiotherapy (CRT) is standard for many patients but is frequently complicated by radiation-induced oral mucositis, a dose-limiting toxicity that impairs nutrition, treatment compliance, and quality of life.[5–10] Acute phase proteins such as C-reactive protein (CRP) may objectively reflect the inflammatory burden associated with mucosal injury. ***Methods***: This prospective observational study enrolled 75 patients with biopsy-proven HNSCC planned for CRT at a tertiary oncology centre (2021–2022). Seventy-two patients completed protocol-defined follow-up. Oral mucositis was graded using the World Health Organization (WHO) scale, and performance status was recorded with the Eastern Cooperative Oncology Group (ECOG) criteria.[10,11] Venous blood samples were collected at baseline (day 0), days 14, 28 and 42 of CRT, and one month after completion. CRP, erythrocyte sedimentation rate (ESR) and total leukocyte count (TLC) were quantified using standard laboratory methods. Longitudinal changes and associations with mucositis grades were analysed; $p < 0.05$ was considered statistically significant. ***Results***: Mucositis developed in all patients during CRT. Grade 1–2 mucositis predominated (day 14: 97.2% grade 1–2; day 28: 98.6% grade 1–2), with only transient grade 3 events (2.8% at day 14, 1.4% at day 28). At one month post-treatment, 20.8% had complete resolution, while 76.4% had residual grade 1 mucositis. Mean CRP rose from 11.21 at baseline to 34.45 at day 42, before falling to 8.40 one month post-CRT ($p < 0.001$). ESR and TLC showed no significant overall variation ($p = 0.15$ and $p = 0.91$, respectively). CRP increased stepwise with mucositis severity, from 10.07 (grade 0) to 93.13 (grade 3). ***Conclusion***: Radiation-induced mucositis was nearly universal among HNSCC patients receiving CRT. CRP behaved as a dynamic, treatment-responsive biomarker closely aligned with mucositis severity, whereas ESR and TLC lacked discriminatory value. Serial CRP assessment may aid in monitoring and potentially anticipating clinically significant mucositis during CRT.

Keywords: Head and neck squamous cell carcinoma; oral mucositis; chemo-radiotherapy; C-reactive protein; acute phase proteins; inflammatory biomarkers.

INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) is a major public health challenge in India, where more than 225,000 new cases and approximately 125,000 deaths are reported annually.[1] The disease is closely linked to tobacco use and typically presents in the sixth and seventh decades of life, often at locally advanced stages.[2,3] Most tumours arise from the mucosal epithelium of the oral cavity, oropharynx, hypopharynx and larynx, reflecting patterns of carcinogen exposure in this region.[3,4] Global cancer

reports highlight the disproportionate burden of head and neck malignancies in low- and middle-income countries, underscoring the importance of optimising both oncologic and toxicity outcomes.[4]

Radiotherapy, delivered either alone or in combination with surgery and/or chemotherapy, forms an essential component of curative treatment for HNSCC.[5] Modern radiotherapy protocols frequently employ concurrent platinum-based chemotherapy to enhance tumour control, but at the cost of increased acute toxicity, particularly mucositis.[5,9,10] Oral mucositis, characterised by erythema, oedema and atrophy of the oral and oropharyngeal mucosa progressing to ulceration, is reported in 85–100% of patients receiving head and neck radiotherapy.[6–8,10] Severe mucositis leads to odynophagia, dysphagia, weight loss and treatment interruptions, adversely affecting disease outcomes and quality of life.[6–10]

The pathobiology of radiation-induced mucositis involves complex interactions between direct epithelial damage, oxidative stress, pro-inflammatory cytokine release and secondary infection.[8,9] These processes trigger a systemic acute phase response driven largely by hepatic synthesis of acute phase proteins (APPs), including C-reactive protein (CRP), serum amyloid A, fibrinogen and ferritin.[8,12,13] Among these, CRP is a widely available and sensitive marker of inflammation whose levels rise rapidly in response to tissue injury.[12,13] ESR and total leukocyte count (TLC) are also commonly used clinical markers of systemic inflammation, though they are less specific.[12,13]

Grading of oral mucositis is clinically important for standardising toxicity reporting, comparing treatment protocols and guiding supportive care.[6,10] The World Health Organization (WHO) mucositis scale integrates both clinical appearance and functional impact (ability to eat and drink) and is widely employed in oncology trials.[10] However, despite standardised clinical scoring, objective laboratory markers that correlate with the onset and severity of mucositis remain incompletely defined. Identifying such biomarkers could allow earlier intervention, personalised supportive care and more informed decisions about treatment continuation or modification.

Previous work has suggested that elevations in CRP may parallel the development of severe mucositis in patients undergoing radiotherapy for head and neck cancer, but the data remain limited, particularly from Indian populations.[12,13] Furthermore, the relative utility of ESR and TLC in this setting has not been clearly established. Against this background, the present study was conducted to quantitatively assess changes in selected acute phase proteins—CRP, ESR and TLC—during and after concurrent chemo-radiation in patients with HNSCC, and to correlate these changes with serially graded mucositis severity and patient performance status.

MATERIALS AND METHODS

Study design and setting

This was a prospective observational study conducted in the Department of Radiation Oncology, Rabindra Nath Tagore Medical College, Udaipur, Rajasthan, India. The study period spanned 2021–2022. The institutional ethics committee approved the protocol, and all participants provided written informed consent prior to enrolment, in accordance with the Declaration of Helsinki.

Participants

Seventy-five consecutive patients with histologically confirmed HNSCC who were planned for concurrent chemo-radiotherapy (CRT) according to departmental protocols were initially enrolled. Patients with recurrent disease or a history of prior radiotherapy or chemotherapy to the head and neck region were excluded. Additional exclusion criteria included refusal of consent or inability to comply with follow-up assessments.

Three patients discontinued treatment or follow-up because of poor compliance and were excluded from the final analysis, leaving 72 evaluable patients. Demographic and disease-related information, including age, sex and primary tumour site, were recorded at baseline.

Treatment protocol

All patients received external beam radiotherapy to the primary tumour and regional lymphatics using standard fractionation schedules, as per departmental policy.[5,10] Radiotherapy was delivered with curative intent, either as definitive CRT or as adjuvant treatment following surgery. Concurrent chemotherapy was administered using institutional cisplatin-based regimens, tailored according to patient performance status and comorbidities.

Assessment of mucositis and performance status

Oral mucositis was assessed clinically at baseline (prior to radiotherapy) and subsequently on days 14, 28 and 42 of CRT, and one month after completion of treatment. Mucositis was graded using the WHO oral mucositis scale, which classifies toxicity from grade 0 (no mucositis) to grade 4 (severe ulceration and alimentation impossible) based on clinical appearance and functional impairment.[10]

General condition and tolerability of treatment were evaluated in parallel using the Eastern Cooperative Oncology Group (ECOG) performance status scale.[11] All assessments were performed by

radiation oncology faculty to minimise inter-observer variability.

Laboratory measurements

Venous blood samples were drawn on day 0 (prior to the first radiotherapy fraction), days 14, 28 and 42 of CRT, and one month after completion of CRT. Samples were processed in the hospital's central laboratory.

Serum CRP levels were measured using standard quantitative methods routinely employed in the institution's biochemistry laboratory. ESR was determined by the Westergren method, and TLC was obtained using an automated haematology analyser. All measurements were performed according to manufacturer instructions and internal quality-control procedures.

Outcomes

The primary objective was to evaluate temporal changes in CRP, ESR and TLC during and after CRT and to examine their association with the severity of radiation-induced oral mucositis. Secondary objectives included describing the distribution and evolution of mucositis grades over time and characterising the relationship of acute phase reactants with mucositis grades.

Statistical analysis

Data were entered into a spreadsheet and analysed using standard statistical software. Continuous variables (CRP, ESR, TLC) were summarised as means. Categorical variables (mucositis grades) were summarised as counts and proportions. Longitudinal changes in CRP, ESR and TLC over the five assessment points were evaluated using appropriate repeated-measures comparisons. Associations between biomarker levels and mucositis grades were assessed using comparative tests for continuous variables across ordered categories. A two-sided p value < 0.05 was considered indicative of statistical significance. In the present dataset, the overall variation in CRP over time and across mucositis grades was highly significant ($p < 0.001$), whereas ESR and TLC did not show statistically significant variation.

RESULTS

Patient and tumour characteristics

Of the 72 evaluable patients, 49 were male and 23 were female. All patients received planned concurrent chemo-radiotherapy. Primary tumour sites are summarised in Table 1. Carcinoma of the tongue formed the largest subgroup (n = 28), followed by buccal mucosa (n = 11) and supraglottic region (n = 8). Less frequent sites included pyriform fossa, lower alveolus, posterior pharyngeal wall and other subsites of the oral cavity and pharynx, reflecting the typical distribution of tobacco-related HNSCC in the region.

Table 1. Classification of patients according to gender and site of involvement of HNSCC

Region	Male	Female	Total
Secondary neck	3	0	3
Supraglottic	7	1	8
Tongue	22	6	28
Posterior pharyngeal wall	1	1	2
Pyriform fossa	7	0	7
Buccal mucosa	9	2	11
Hard palate	1	0	1
Lower alveolus	1	2	3
Oropharynx	1	0	1
Tonsil	1	0	1
Laryngopharynx	1	0	1
Retromolar triagonal	1	0	1
Soft palate	1	0	1
Larynx	1	0	1

Table 1 shows a clear male predominance among patients with HNSCC undergoing concurrent chemo-radiotherapy, consistent with known sex differences in tobacco and alcohol exposure.The tongue and buccal mucosa together account for the majority of primary sites, supporting the recognised burden of oral cavity cancers in Indian populations. Less common tumours involve the supraglottic larynx, pyriform fossa and other upper aerodigestive subsites, reflecting the heterogeneous but predominantly mucosal distribution typical of HNSCC.

Evolution of oral mucositis during and after treatment

The incidence and severity of mucositis over time are presented in Table 2. No patient had mucositis at baseline. By day 14 of CRT, all 72 patients had developed mucositis, predominantly grade 1 (n = 42) or grade 2 (n = 28), with 2 patients experiencing grade 3 mucositis. On day 28, grade 1 mucositis persisted in 39 patients, grade 2 in 32 patients, and grade 3 in 1 patient. By day 42, toxicity had shifted towards milder grades, with 60 patients having grade 1 and 12 patients grade 2 mucositis; no grade 3 events were observed. One month after completion of CRT, 15 patients showed complete resolution of mucositis (grade 0), 55 had residual grade 1 mucositis, and only 2 had grade 2 mucositis. The overall change in mucositis severity across time points was highly significant ($p < 0.001$).

Table 2. Incidence of various grades of mucositis during follow-up

Mucositis grade	Day 14	Day 28	Day 42	1 month after completion
Grade 0	0	0	0	15
Grade 1	42	39	60	55
Grade 2	28	32	12	2
Grade 3	2	1	0	0

Table 2 demonstrates that radiation-induced mucositis emerged early during concurrent chemo-radiotherapy, with all patients affected by day 14. The distribution shifted over time, with a peak in grade 2–3 mucositis around days 14–28, followed by gradual de-escalation towards predominantly grade 1 mucositis by day 42 and at one month post-treatment. Complete resolution occurred in approximately one-fifth of patients at one month. The highly significant p value highlights a dynamic, treatment-related pattern of mucosal toxicity.

Temporal profile of CRP, ESR and TLC

Mean values of CRP, ESR and TLC at each assessment point are shown in Table 3. Baseline mean CRP was 11.21 and rose progressively to 20.65 at day 14, 17.20 at day 28 and 34.45 at day 42, before falling to 8.40 one month after completion of CRT. This overall variation was highly significant ($p < 0.001$). ESR increased modestly from 21.04 at baseline to 27.22 at day 14, then declined slightly and stabilised around 24–25 thereafter; overall variation was not statistically significant ($p = 0.15$). TLC showed minor fluctuations without significant change over time ($p = 0.91$).

Table 3. Mean values of CRP, ESR and TLC during follow-up and by mucositis grade

By follow-up day

Days of follow-up	CRP (Mean)	ESR (Mean)	TLC (Mean)
0 days (baseline)	11.21	21.04	6829.59
14 days	20.65	27.22	6636.25
28 days	17.20	24.27	6842.37
42 days	34.45	24.51	6957.69
1 month after completion	8.40	24.87	6869.27

By mucositis grade

Mucositis grade	CRP (Mean)	ESR (Mean)	TLC (Mean)
Grade 0	10.07	21.62	6770.83
Grade 1	15.23	24.40	6875.37
Grade 2	33.50	27.62	6773.68
Grade 3	93.13	23.67	6616.00

Table 3 shows that CRP behaved as a dynamic acute phase reactant, rising steadily during chemo-radiotherapy with a peak at day 42 and returning to below baseline levels one month after treatment completion. In contrast, ESR exhibited only modest and statistically non-significant fluctuations, while TLC remained largely stable, suggesting limited sensitivity to mucosal inflammation. When stratified by mucositis grade, CRP increased markedly from grades 0–3, whereas ESR and TLC did not display a consistent relationship with toxicity severity.

Relationship between acute phase proteins and mucositis severity

Comparison of CRP values across mucositis grades showed a strong positive association: mean CRP values increased from 10.07 in patients without mucositis (grade 0) to 15.23 in grade 1, 33.50 in grade 2 and 93.13 in grade 3 mucositis (Table 3). This trend was highly significant ($p < 0.001$). ESR values tended to be higher in grade 2 mucositis (27.62) but did not show a statistically significant association with severity ($p = 0.07$). TLC varied minimally across grades ($p = 0.96$), indicating that leukocyte count alone may be a poor discriminator of mucositis burden.

Figures

FIGURE 1. TEMPORAL TREND IN MEAN CRP LEVELS DURING AND AFTER CHEMO-RADIOTHERAPY

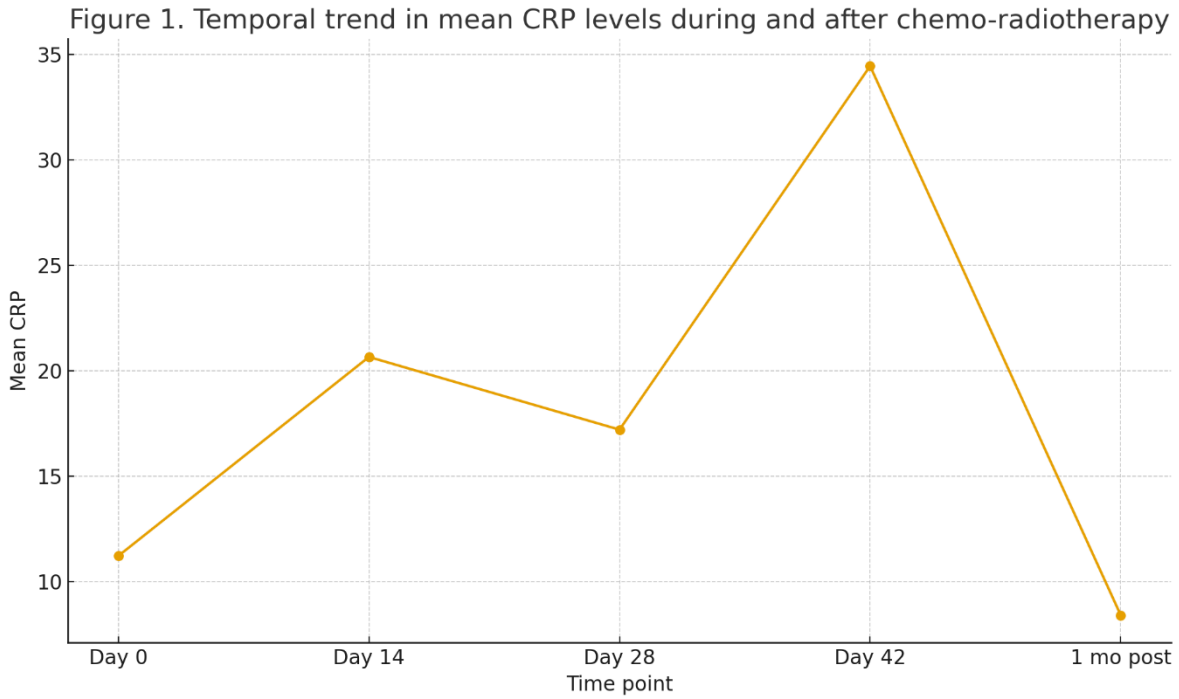

Figure 1 illustrates a characteristic acute phase pattern of CRP during the treatment course. Mean CRP values rose from a relatively low baseline to higher levels by day 14, dipped slightly at day 28, and then peaked at day 42, coinciding with maximal cumulative radiation exposure. One month after treatment completion, CRP declined to below baseline, paralleling clinical recovery of the oral mucosa. This pattern reinforces CRP as a sensitive, reversible marker of CRT-related inflammatory burden.

FIGURE 2. DISTRIBUTION OF MUCOSITIS GRADES AT EACH ASSESSMENT POINT

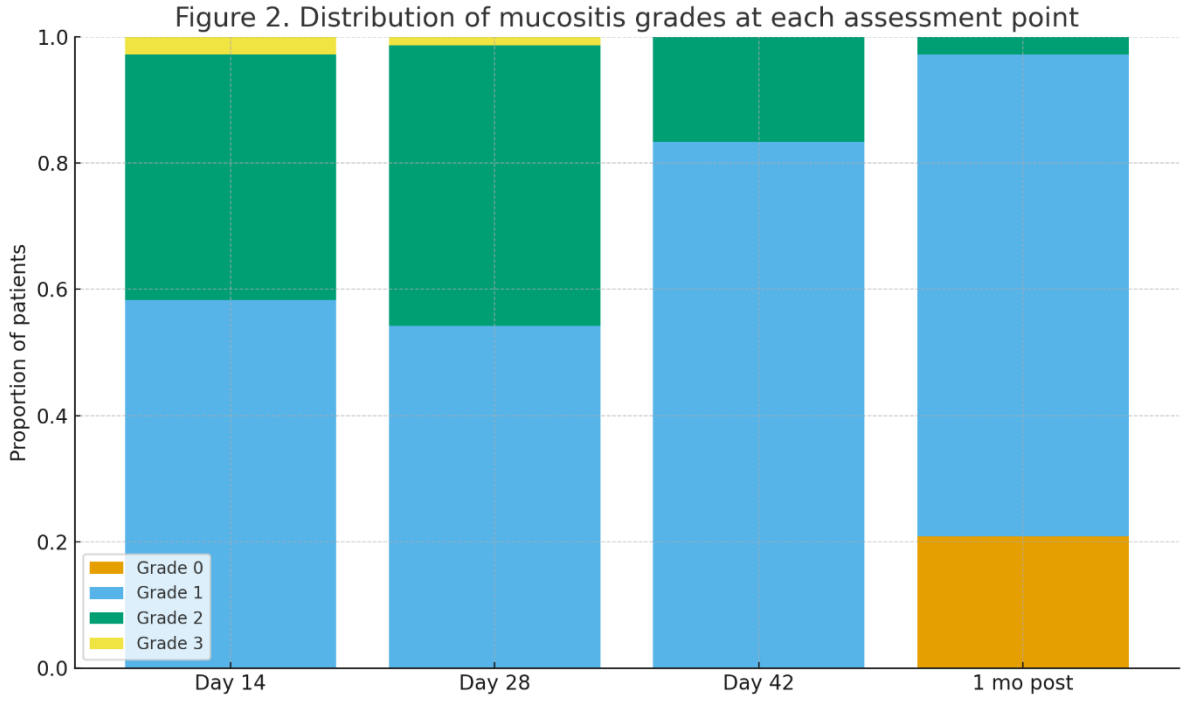

Figure 2 summarises the shifting spectrum of mucositis severity over time. Early during CRT, there is a broad

distribution with substantial proportions of both grade 1 and grade 2 mucositis, and a small number of grade 3 cases. As treatment progresses and subsequently concludes, the distribution narrows towards predominantly grade 1 toxicity, with resolution to grade 0 in a subset by one month post-therapy. These visual trends complement the tabulated data, emphasising both the inevitability and reversibility of mucosal injury.

DISCUSSION

This prospective study examined serial changes in selected acute phase reactants and their relationship with radiation-induced mucositis in patients with HNSCC receiving concurrent chemo-radiotherapy. Nearly all patients developed clinically significant mucositis, predominantly of grade 1–2 severity, and CRP emerged as a robust biomarker that closely mirrored both treatment phase and mucositis grade, whereas ESR and TLC did not show meaningful associations.

The near-universal occurrence of mucositis observed here is consistent with the reported 85–100% incidence in contemporary head and neck radiotherapy protocols.[6,8–10] Our distribution of grade 1 (54.4%) and grade 2 (20.5%) mucositis is very similar to that described by Chethana et al., who reported 54% and 19% for grades 1 and 2, respectively, though their proportion of grade 3 mucositis was slightly higher (3% vs. 0.8%).[12] By contrast, Ki et al. observed a higher burden of moderate-to-severe mucositis (grades 2–3 in 90% of patients), likely reflecting differences in treatment regimens, target volumes or supportive care practices.[13] The relatively low incidence of persistent grade 3 toxicity in our cohort may be attributable to meticulous oral care and careful treatment monitoring.

Our findings reinforce the central role of CRP as an objective marker of treatment-related inflammation. Mean CRP levels increased significantly during CRT, peaking towards the end of treatment before declining to sub-baseline values one month after completion. This trajectory closely parallels the clinical course of mucositis and corroborates observations by Ki et al., who reported a similarly significant association between CRP elevation and the development of severe mucositis in head and neck cancer patients receiving radiotherapy.[13] Chethana et al. also documented significant dynamic changes in acute phase proteins, including CRP, during post-chemo-radiation mucositis.[12]

The stepwise rise in CRP across mucositis grades in our study—from grade 0 through grade 3—further supports its utility as a dose-response biomarker of mucosal injury.[8,12,13] This pattern is biologically plausible, given that epithelial damage from ionising radiation initiates a cascade of pro-inflammatory cytokines that stimulate hepatic synthesis of CRP and other APPs.[8,9] Elevated CRP may therefore capture both local mucosal inflammation and systemic inflammatory responses triggered by treatment and associated infections.

In contrast, ESR and TLC showed only minor, statistically non-significant fluctuations over time and across mucositis grades. Our findings align with Chethana et al., who did not observe a strong relationship between ESR or leukocyte counts and mucositis severity.[12] Ki et al. similarly reported limited discriminatory value of ESR in predicting radiation-induced mucositis.[13] ESR is influenced by numerous non-inflammatory factors, including anaemia and plasma protein composition, while TLC reflects broader immune status rather than specific mucosal injury, which may explain their limited performance as targeted toxicity markers.

Clinically, the ability to track CRP as a quantitative adjunct to clinical mucositis grading has several potential implications. Elevated or rapidly rising CRP during CRT could prompt intensification of oral hygiene measures, early nutritional support, more aggressive analgesia or prophylactic antimicrobials, with the goal of preventing progression to functionally debilitating mucositis.[6–9,12,13] In selected high-risk patients, trends in CRP might even inform decisions about brief treatment breaks or modifications, although such strategies require validation in prospective trials.[5,10]

This study has limitations. It was conducted at a single institution with a modest sample size, which may limit generalisability. We evaluated only three readily available markers—CRP, ESR and TLC—without concurrent measurement of cytokines or other novel biomarkers. Mucositis was assessed clinically using the WHO scale but did not incorporate patient-reported quality-of-life instruments.[6,10] Finally, treatment techniques and chemotherapy schedules, while reflective of real-world practice, were not stratified in detail, which could influence toxicity patterns.

Despite these limitations, the study adds to the growing body of evidence that CRP is a sensitive, clinically accessible biomarker of radiation-induced mucositis in HNSCC.[8,12,13] Future research should explore composite biomarker panels, integration with advanced radiotherapy dosimetric parameters and evaluation of CRP-guided supportive care algorithms in larger, multicentre cohorts.

CONCLUSION

In this prospective cohort of patients with head and neck squamous cell carcinoma undergoing concurrent

chemo-radiotherapy, radiation-induced oral mucositis was almost universal and predominantly of grade 1–2 severity. C-reactive protein behaved as a dynamic acute phase reactant, rising in parallel with treatment intensity and mucositis severity and normalising after therapy, while ESR and TLC provided limited additional information. These findings support the use of serial CRP measurement as an objective adjunct to clinical mucositis grading, with potential to refine toxicity monitoring and guide supportive care. Larger studies are warranted to validate CRP-based risk stratification and to integrate inflammatory biomarkers into personalised head and neck cancer treatment pathways.

REFERENCES

1. International Agency for Research on Cancer. Globocan 2020 India (Internet). Global Cancer Observatory. 2021 [cited 3/2021]. Available from: https://gco.iarc.fr.
2. Gupta PC. Mouth cancer in India: a new epidemic? *J Indian Med Assoc.* 1999 Sep;97(9):370-3.
3. Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. *CA Cancer J Clin.* 2007 Jan-Feb;57(1):43-66.
4. Boyle P, Levin B. World Cancer Report 2008. International Agency for Research on Cancer (IARC). Geneva: IARC; 2008. p. 170.
5. Fu KK, et al. A Radiation Therapy Oncology Group (RTOG) phase III randomized study to compare hyperfractionation and two variants of accelerated fractionation to standard fractionation radiotherapy for head and neck squamous cell carcinomas: first report of RTOG 9003. *Int J Radiat Oncol Biol Phys.* 2000;48(1):7-30.
6. Parulekar W, Mackenzie R, Bjarnason G, Jordan RC. Scoring oral mucositis. *Oral Oncol.* 1998 Jan;34(1):63-71.
7. Raber-Durlacher JE, Elad S, Barsch A. International Classification of Orofacial Pain, 1st edition (ICOP). *Cephalalgia.* 2020;40(2):129-221.
8. Shih A, Miaskowski C, Dodd MJ, et al. Mechanisms of radiation-induced oral mucositis and the consequences. *Cancer Nurs.* 2003;26(3):222-229.
9. Vera-Llonch M, Oster G, Hagiwara M, Sonis S. Oral mucositis in patients undergoing radiation treatment for head and neck carcinoma. *Cancer.* 2006 Jan 15;106(2):329-36.
10. Trotti A, Bellm LA, Epstein JB, Frame D, Fuchs HJ, Gwede CK, et al. Mucositis incidence, severity and associated outcomes in patients with head and neck cancer receiving radiotherapy with or without chemotherapy: a systematic literature review. *Radiother Oncol.* 2003 Mar;66(3):253-62.
11. Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. *Am J Clin Oncol.* 1982 Dec;5(6):649-55.
12. Chethana, Rao PS, Madathil LP, Rao S, Shetty P, Patidar M. Quantitative analysis of acute phase proteins in post chemo-radiation mucositis. *J Clin Diagn Res.* 2015 Oct;9(10):ZC28-31.
13. Ki Y, Kim W, Nam J, Kim D, Park D, Kim D. C-reactive protein levels and radiation-induced mucositis in patients with head-and-neck cancer. *Int J Radiat Oncol Biol Phys.* 2009 Oct 1;75(2):393-8.

