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Impaired urease-mediated alkali generation following radiotherapy in head and neck cancer patients: a pilot salivary biochemical analysis

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Abstract

Introduction

Radiotherapy for head and neck cancers is associated with significant alterations in the oral environment, including salivary dysfunction. Urease-mediated hydrolysis of urea represents a key alkalinogenic pathway that contributes to plaque pH homeostasis. However, its behaviour across different stages of radiotherapy remains insufficiently explored. This study aimed to evaluate salivary urea concentration and urease activity in patients undergoing radiotherapy and to assess their potential role in altering oral biochemical balance.

Methods

48 patients were divided into four groups (n = 12 each): healthy controls, pre-radiotherapy patients, early post-radiotherapy patients (< 3 months), and late post-radiotherapy patients (> 6 months). Salivary urea concentration was measured using a colorimetric assay, and urease activity was assessed using a urea depletion method following incubation at 37°C for 60 minutes. Statistical analysis was conducted using one-way ANOVA followed by Tukey's post-hoc test, with significance set at $p < 0.05$.

Results

Mean salivary urease activity varied significantly among study groups (one-way ANOVA: $F = 6.271$, $p = 0.002$). Observed differences across groups: the lowest urease activity was recorded in the early post-radiotherapy group (< 3 months) (0.179 ± 0.095). The early post-radiotherapy group exhibited the highest mean urea concentration (26.711 ± 12.454 mMol/L).

Conclusion

Low urease activity post-radiotherapy indicates a reduced alkaline environment of the oral cavity. While no direct assessment of dental caries was performed, these biochemical alterations may contribute to conditions favourable for plaque acidification and subsequent caries development.

Introduction

Head and neck cancers pose a substantial global health challenge, with radiotherapy serving as a key treatment for these malignancies. Despite progress in therapeutic approaches, radiotherapy is linked to a broad spectrum of oral complications that can greatly affect patients' quality of life. Among these, radiation-induced caries (RIC) is recognised as one of the most aggressive and rapidly advancing forms of dental destruction, often causing extensive tooth loss within months of treatment. [1,2] These lesions

have a distinct clinical appearance, usually affecting cervical regions and incisal edges, and differ markedly from traditional caries in their pattern and progression.[3]

The pathogenesis of radiation-induced caries is multifactorial and remains only partially understood. Traditionally, xerostomia resulting from radiation-damaged salivary glands has been regarded as the primary cause.[4,5] Radiotherapy damages the structure and function of salivary glands, resulting in reduced salivary flow, impaired buffering capacity, and changes in salivary composition.[6] Since saliva plays a vital role in maintaining oral health through mechanical cleansing, antimicrobial activity, and remineralisation, its decrease increases the risk of rapid cariogenic challenges. [6,7] However, the severity and swift progression of RIC cannot be fully explained by hyposalivation alone, suggesting the involvement of additional biochemical and microbial factors. [2,8]

Dental caries is a biofilm-mediated disease resulting from ecological shifts within the oral microbiome. According to the ecological plaque hypothesis, the oral microbial community exists in a dynamic equilibrium with the host, and environmental perturbations such as frequent acid exposure or reduced salivary flow can disrupt this balance, favouring acidogenic and aciduric microorganisms.[9–11] These bacteria metabolise dietary carbohydrates to produce organic acids, thereby sustaining plaque acidification and enamel demineralisation.[12] The extended ecological hypothesis further emphasises that disease progression is governed by the balance between demineralisation and protective mechanisms within the biofilm.[13]

While acid production has long been considered central to the caries process, increasing attention has been directed toward the role of alkalinogenic pathways in maintaining plaque pH homeostasis. Alkali generation acts as a natural defence mechanism by neutralising acids and stabilising the oral environment. [14,15] Among these pathways, urease-mediated hydrolysis of urea is particularly important. Urease-producing bacteria hydrolyse salivary urea into ammonia and carbon dioxide, thereby increasing plaque pH and inhibiting acidogenic microbial activity. [16]

The significance of urease activity in caries prevention has been well documented. Studies have demonstrated that individuals with low caries experience exhibit higher urease and arginine deiminase system activity compared with caries-active individuals.[17,18] Additionally, increased ammonia production from urea metabolism has been associated with improved regulation of plaque pH and reduced caries risk. [19] Longitudinal and prospective studies further suggest that reduced urease activity may be a significant risk factor for caries development due to impaired alkalinogenic potential within dental biofilms. [20]

The balance between acidogenic and alkalinogenic activities is therefore critical in maintaining oral ecological stability. Disruption of this balance results in a shift toward a cariogenic biofilm characterised by persistent acidification and enhanced demineralisation.[11,14] Importantly, this imbalance may be

exacerbated in patients undergoing radiotherapy due to concurrent alterations in salivary composition and microbial ecology.

Radiotherapy has been shown to induce significant changes in the oral environment beyond salivary hypofunction. Alterations in salivary proteins, enzymes, and metabolites, along with shifts in microbial composition, contribute to dysbiosis within dental biofilms.[4,21] Furthermore, an increase in acidogenic and aciduric bacterial species, including Streptococcus mutans and Lactobacillus, has been reported following radiotherapy, further promoting a cariogenic environment. [22] Epidemiological evidence highlights the clinical significance of radiation-induced caries. Systematic reviews report that approximately 29–37% of patients develop dental caries following head and neck radiotherapy. [23] In addition to its high prevalence, RIC is associated with increased treatment complexity, reduced oral function, and diminished quality of life. [2,3]

Saliva has emerged as a valuable diagnostic medium due to its non-invasive collection and its ability to reflect both local and systemic physiological changes. Salivary biomarkers (functional, microbial, physico-chemical) provide insights into biochemical and microbial alterations within the oral environment and have been increasingly explored for caries detection and disease monitoring.[24,25] Despite the established importance of urease-mediated alkalinogenic pathways in oral health, their role in radiation-induced oral alterations remains largely unexplored. Most existing studies have focused on salivary gland dysfunction and structural changes in dental tissues as primary contributors to RIC.[2,3,5]

The present pilot study was therefore designed to assess salivary urea concentration and urease activity at various stages of radiotherapy in patients with head and neck cancer. The null hypothesis stated that there is no difference in salivary urease activity between patients with or without radiotherapy.

Methods

Study Design

The present study was designed as a cross-sectional analytical pilot investigation to evaluate salivary urea concentration and urease activity in patients with head and neck cancer undergoing radiotherapy. The study protocol adhered to the STROBE guidelines for observational studies. Out of a total of 60 patients assessed, 48 were recruited and categorised into four groups, with 12 individuals in each group: Healthy control group; Pre-radiotherapy head and neck cancer patients; Early post-radiotherapy patients (< 3 months after completion of radiotherapy); Late post-radiotherapy patients (> 6 months after completion of radiotherapy) Participants were recruited from patients receiving treatment for head and neck cancer at the radiation oncology department. Healthy individuals without systemic disease or undergoing current cancer therapy were included as controls (Fig. 1).

Inclusion criteria included patients aged 18 years and above, diagnosed with head and neck cancer, undergoing radiotherapy, possessing at least one natural tooth, and willing to provide informed consent.

Patients with systemic conditions affecting salivary flow (e.g., Sjögren's syndrome), those who received antibiotics within the previous month, individuals who had recent dental treatment or active oral infections, and those with a history of chemotherapy-induced salivary gland disorders unrelated to radiotherapy were excluded from the study. Written informed consent was taken from the participants who were finally recruited for the study.

Saliva Collection

Unstimulated whole saliva samples were collected using the passive drool method. Sample collection was performed between 8:00 AM and 11:00 AM to minimise circadian variation in salivary composition. Participants were instructed to refrain from eating, drinking, smoking, or performing oral hygiene procedures for at least 90 minutes prior to sample collection. Approximately 2–3 mL of saliva was collected from each participant into sterile containers. The collected samples were immediately transported to the laboratory and centrifuged at 10,000 rpm for 15 minutes to remove cellular debris. The supernatant was then separated and stored at − 20°C until further biochemical analysis.

Biochemical Analysis

Salivary urea concentration was measured using a commercially available colorimetric urea assay kit according to the manufacturer's instructions. To assess urease activity, a urea depletion method was employed. Baseline urea concentration was measured immediately after saliva collection. A second measurement was obtained after incubating the saliva samples at 37°C for 60 minutes. The reduction in urea concentration between the two measurements was considered indicative of urease-mediated urea metabolism. All assays were performed in duplicate, and the mean value was used for statistical analysis to ensure the results were reliable.

Analytical Reliability of Biochemical Measurements

All salivary samples underwent duplicate biochemical analysis, and selected samples were evaluated in triplicate to ensure analytical precision and reproducibility. Mean values derived from replicate measurements were used for all statistical analyses to minimise assay variability and avoid pseudo-replication. Replicate testing demonstrated consistent assay performance across study groups.

Statistical Analysis

Data analysis was performed using appropriate statistical software. The normality of data distribution was assessed using the Shapiro–Wilk test. Intergroup comparisons were conducted using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons. A p-value < 0.05 was considered statistically significant.

Results

Participant Characteristics

After screening 60 patients, 48 participants were included in this cross-sectional pilot study and divided into four groups: healthy controls, pre-radiotherapy patients, early post-radiotherapy (< 3 months), and late post-radiotherapy (> 6 months), with 12 participants in each group. The study population predominantly consisted of middle-aged individuals diagnosed with head and neck cancer, reflecting the demographic commonly undergoing radiotherapy. The mean age increased progressively across radiotherapy stages, ranging from 41.2 ± 7.4 years in controls to 52.1 ± 6.2 years in the late post-radiotherapy group. Male participants were slightly more prevalent in cancer cohorts.

Radiotherapy doses were comparable between post-radiotherapy groups, averaging approximately 64–66 Gy. Body mass index and smoking distribution showed no major variation among groups (Table 1).

Table 1
Demographic and clinical characteristics of participants

Variable	Controls (n = 12)	Pre-RT (n = 12)	< 3 Months Post-RT (n = 12)	> 6 Months Post-RT (n = 12)
Age (years, mean ± SD)	41.2 ± 7.4	47.8 ± 6.9	50.5 ± 5.8	52.1 ± 6.2
Sex (M/F)	6/6	8/4	9/3	8/4
Mean RT Dose (Gy)	—	—	64.3 ± 3.8	65.7 ± 4.1
Smokers (n)	1	4	5	4
BMI (kg/m²)	23.8 ± 2.1	22.9 ± 2.4	22.4 ± 2.0	22.7 ± 1.9

Salivary Urease Activity

Mean salivary urease activity varied significantly among study groups (one-way ANOVA: $F = 6.271$, $p = 0.002$). The control group showed the highest urease activity (0.453 ± 0.214). Decreased activity was observed after radiotherapy, with the lowest urease activity recorded in the early post-radiotherapy group (< 3 months) (0.179 ± 0.095). The pre-radiotherapy and late post-radiotherapy groups showed intermediate values (Table 2).

Table 2
Comparison of Salivary Urease Activity Across Groups

Group	Mean	SD	Range(Min–Max)
Control	0.453	0.214	0.28–0.88
Pre-RT	0.306	0.097	0.16–0.44
< 3 Months Post-RT	0.179	0.095	0.07–0.37
> 6 Months Post-RT	0.295	0.128	0.11–0.44
One-way ANOVA; **F = 6.271, p = 0.002**			

Post-hoc Tukey's test revealed a statistically significant reduction in controls compared with early post-radiotherapy patients (mean difference = 0.274, p = 0.001). Although urease activity increased beyond six months after radiotherapy, values remained lower than those in healthy controls (Fig. 2).

Salivary Urea Concentration

Salivary urea concentration demonstrated significant variation across radiotherapy stages (F = 7.051, p = 0.001). The early post-radiotherapy group exhibited the highest mean urea concentration (26.711 ± 12.454 mMol/L), markedly greater than that observed in controls (9.897 ± 3.453 mMol/L). Pre-radiotherapy and late post-radiotherapy groups showed intermediate concentrations (Table 3).

Table 3
Salivary Urea Concentration Across Radiotherapy Stages

Group	Mean (mMol/L)	SD	Range(Min–Max)
Control	9.897	3.453	4.39–13.87
Pre-RT	14.044	5.135	8.84–24.33
< 3 Months Post-RT	26.711	12.454	10.42–54.75
> 6 Months Post-RT	16.730	9.839	8.85–36.10
ANOVA; F = 7.051, p = 0.001			

Pairwise comparisons indicated significant differences between controls and early post-radiotherapy patients (p < 0.001) and between the early post-radiotherapy and pre-radiotherapy groups (p = 0.011). A reduction in urea levels was noted in the late post-radiotherapy stage, suggesting biochemical alterations (Fig. 3).

Combined Biochemical Trends Across Radiotherapy Stages

Evaluation of both biochemical parameters revealed an inverse pattern during the early post-radiotherapy phase. While salivary urea concentration reached peak levels during this period, urease activity simultaneously demonstrated maximal suppression. Subsequent stages showed partial normalisation of both parameters; however, the values remained altered relative to those of healthy controls.

Discussion

Radiotherapy remains a cornerstone in the management of head and neck malignancies; however, it is frequently associated with several oral complications that significantly affect patients' quality of life. Among these, radiation-induced dental changes are recognised as particularly aggressive and rapidly progressing forms of tooth destruction [1–3]. These lesions commonly occur on the cervical or incisal surfaces and may lead to rapid tooth breakdown within months of radiotherapy. The pathogenesis of these changes is multifactorial and involves salivary gland dysfunction, structural alterations of dental tissues, and ecological shifts within oral microbial communities.

One of the most significant consequences of head-and-neck radiotherapy is damage to salivary glands. Radiation exposure results in acinar cell degeneration and glandular fibrosis, leading to hyposalivation and xerostomia [4, 21]. Because saliva plays a critical role in buffering plaque acids, controlling microbial growth, and facilitating enamel remineralisation, a reduction in salivary flow substantially increases susceptibility to oral complications [16, 18]. In addition to reduced salivary flow, radiotherapy has also been associated with qualitative alterations in salivary composition. Metabolomic studies have demonstrated that radiotherapy induces significant changes in salivary metabolic pathways, which may disrupt oral ecological balance and contribute to disease progression [26, 27].

Dental caries is widely accepted as a biofilm-mediated disease resulting from ecological disturbances within dental plaque communities. According to the ecological plaque hypothesis, the oral microbiota normally exists in a stable symbiotic relationship with the host; however, environmental disturbances such as reduced salivary flow or repeated acid exposure can disrupt this balance and favour the proliferation of acidogenic microorganisms [9–11]. These organisms metabolise dietary carbohydrates and produce organic acids that lower plaque pH and initiate enamel demineralisation [11, 12]. However, contemporary understanding emphasises that disease progression is governed not only by acid production but also by the balance between acidogenic and protective mechanisms within the biofilm [14, 28].

While acid production has long been considered central to the caries process, increasing evidence highlights the importance of alkali generation within dental biofilms as a protective mechanism against plaque acidification [10, 11, 33]. Among the alkalinogenic pathways present in dental plaque, urease-mediated hydrolysis of urea is particularly important. Urease-producing bacteria convert salivary urea into ammonia and carbon dioxide, thereby increasing plaque pH and counteracting acidogenic bacterial activity [11–13, 33].

Several studies have demonstrated that higher urease activity is associated with improved regulation of plaque pH and enhanced resistance to acidogenic stress, whereas reduced urease activity may impair alkali generation and compromise buffering capacity within dental biofilms [12–15].

A key finding of the present study was elevated salivary urea concentrations, accompanied by significantly reduced urease activity, in the early post-radiotherapy group. This suggests a functional dissociation between substrate availability and microbial metabolic utilisation. Although salivary urea may accumulate due to altered salivary gland function, the microbial pathways responsible for urea metabolism appear to be impaired following radiotherapy.

This imbalance has important mechanistic implications. Under normal conditions, urea serves as a substrate for urease-producing bacteria, facilitating the generation of ammonia and the buffering of plaque acids. In contrast, the present findings indicate that despite increased substrate availability, the enzymatic pathway responsible for alkali generation is compromised. This results in diminished alkalinogenic potential and may contribute to persistence of a more acidic oral environment, thereby promoting ecological imbalance within dental biofilms.

Radiotherapy has been shown to alter oral microbial composition, including reductions in beneficial commensal bacteria and increased colonisation by acidogenic microorganisms such as Streptococcus mutans and Lactobacillus species [41, 44]. These microbial shifts may reduce the prevalence of urease-producing bacteria within dental plaque, thereby decreasing ammonia production and impairing plaque buffering capacity. Consequently, disruption of the balance between acid production and alkali generation may lead to persistent plaque acidification and enhanced susceptibility to ecological imbalance [33, 41].

Another observation in this study was an increase in urease activity in the late post-radiotherapy group. Although urease levels did not fully return to those observed in healthy controls, the observed improvement suggests gradual adaptation of the oral microbial ecosystem over time following completion of radiotherapy. This aligns with emerging evidence indicating resilience and adaptive capacity of the oral microbiome following environmental disturbances [42].

While xerostomia has traditionally been considered the principal factor underlying radiation-associated oral changes, the findings of this study suggest that disruption of microbial alkalinogenic mechanisms may also play an important role. In particular, impairment of urease-mediated pathways represents a previously underexplored aspect of oral biochemical alteration following radiotherapy.

Taken together, the results of this study indicate that radiotherapy is associated with a metabolic imbalance characterised by increased substrate availability (urea) and reduced enzymatic activity (urease). This disruption of urease-mediated alkali generation may contribute to a persistently acidic oral environment and altered microbial homeostasis.

From a clinical perspective, this highlights the need for early preventive strategies and targeted interventions in patients undergoing head and neck radiotherapy. Approaches such as pre-radiotherapy dental assessment, fluoride-based preventive protocols, and salivary substitutes remain essential; however, the present findings also underscore the potential value of therapies that enhance alkalinogenic activity, including arginine-based formulations and probiotics targeting urease-producing bacteria. Additionally, long-term monitoring of salivary function and biochemical markers may help identify high-risk individuals. These insights support a shift toward ecology-driven, mechanism-based preventive care in managing radiation-associated oral complications.

Limitations

Although the present study provides important preliminary insights, certain limitations must be acknowledged. The study was conducted as a pilot investigation with a limited sample size, and urease activity was evaluated using biochemical methods rather than direct microbial analysis. In addition, no clinical assessment of dental caries was performed; therefore, any inference regarding clinical outcomes remains indirect. Future studies incorporating microbial sequencing, larger patient cohorts, and clinical indices are required to further elucidate the relationship between urease activity and oral health outcomes.

Nevertheless, these findings highlight the potential importance of urease-mediated alkalinogenic pathways in maintaining oral ecological balance following radiotherapy and suggest that therapeutic strategies aimed at restoring microbial pH homeostasis may help mitigate radiation-associated oral complications.

Conclusion

Within the limitations of this pilot study, significant alterations in salivary urea concentration and urease activity were observed in patients undergoing radiotherapy. The early post-radiotherapy phase was characterised by elevated urea levels and reduced urease activity, indicating a functional dissociation between substrate availability and microbial enzymatic utilisation, suggesting impaired alkalinogenic potential.

This imbalance reflects a disturbance in oral biochemical homeostasis, which may contribute to a shift toward a more acidic oral environment and altered microbial dynamics following radiotherapy. The findings highlight the potential role of urease-mediated pathways in maintaining oral ecological balance and underscore the importance of microbial metabolic function in post-radiotherapy oral changes.

Although direct clinical assessment of dental caries was not performed, these biochemical alterations provide insight into a potential mechanistic pathway underlying oral ecological imbalance in irradiated patients. Further longitudinal studies incorporating clinical caries indices and microbiome analysis are required to establish definitive clinical correlations and to evaluate the translational relevance of these findings.

Declarations

Statement of Ethics

This study was performed in accordance with the Declaration of Helsinki.

This human study was approved by the Institutional Ethics Committee, King George's Medical College, Lucknow- approval: Ref. Code:42nd-PGTSC-IIA/P119.

All adult participants provided written informed consent to participate in this study.

Consent to participate statement

Written informed consent was taken from the patients who participated in the study

Conflict of Interest Statement

“The authors have no conflicts of interest to declare.”

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Author Contribution

Rhythm Bains: Conceptualisation, supervision of manuscript draft; Pranjali Singh: preparation of manuscript draft, clinical investigations; Mohammad Kaleem Ahmad: Biochemical analysis; Mrinalini Verma: procurement of study subjects; Promila Verma: supervision of manuscript and data

Data Availability

All data supporting the findings of this study are available within the paper. Further enquiries can be directed to the corresponding author

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Figures

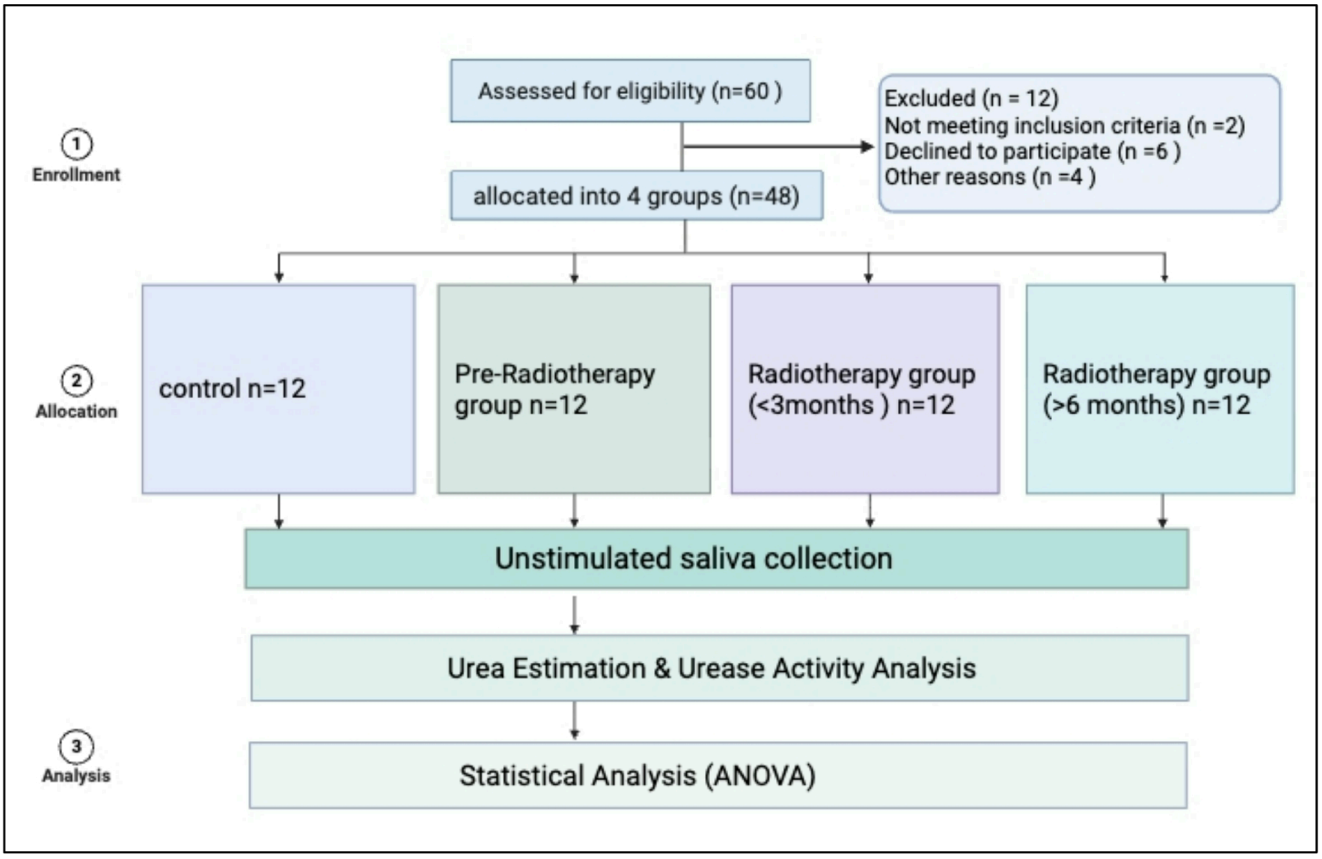


Figure 1

STROBE Flowchart

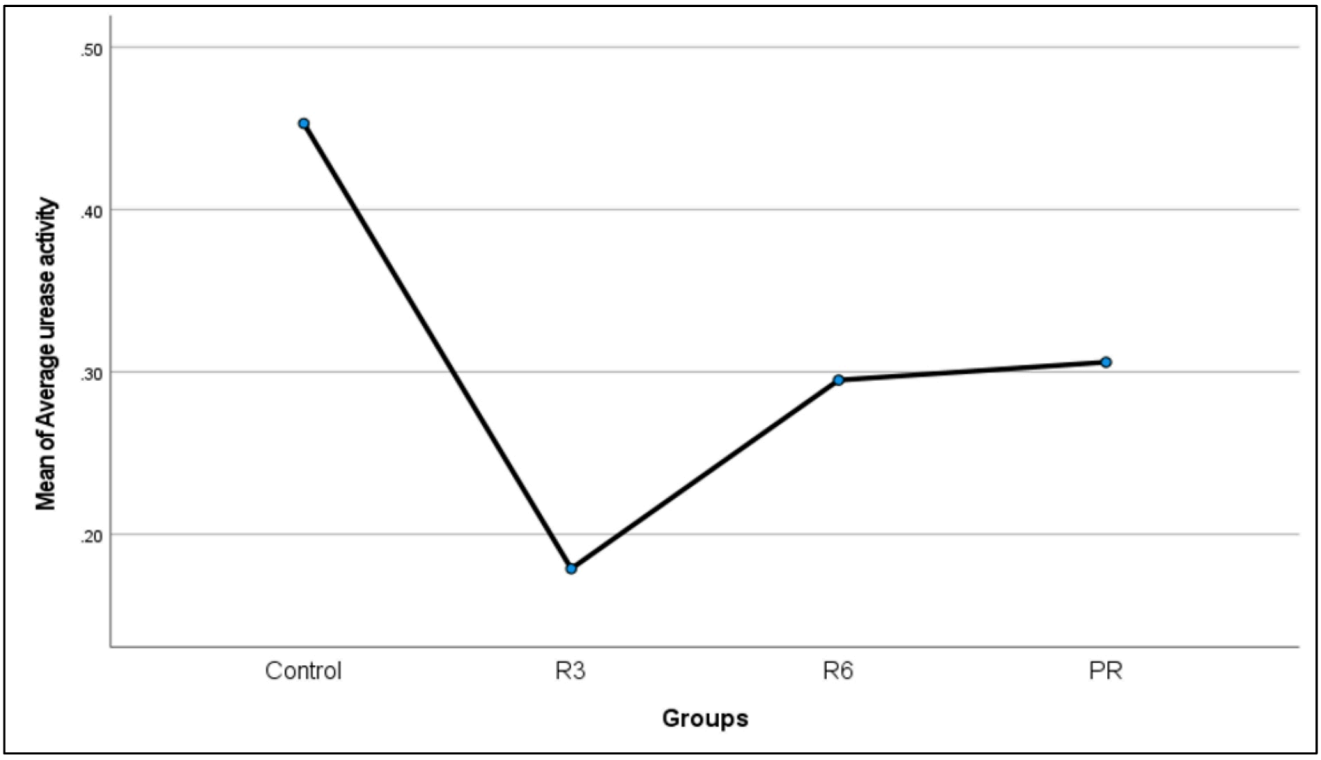


Figure 2

Average urease activity

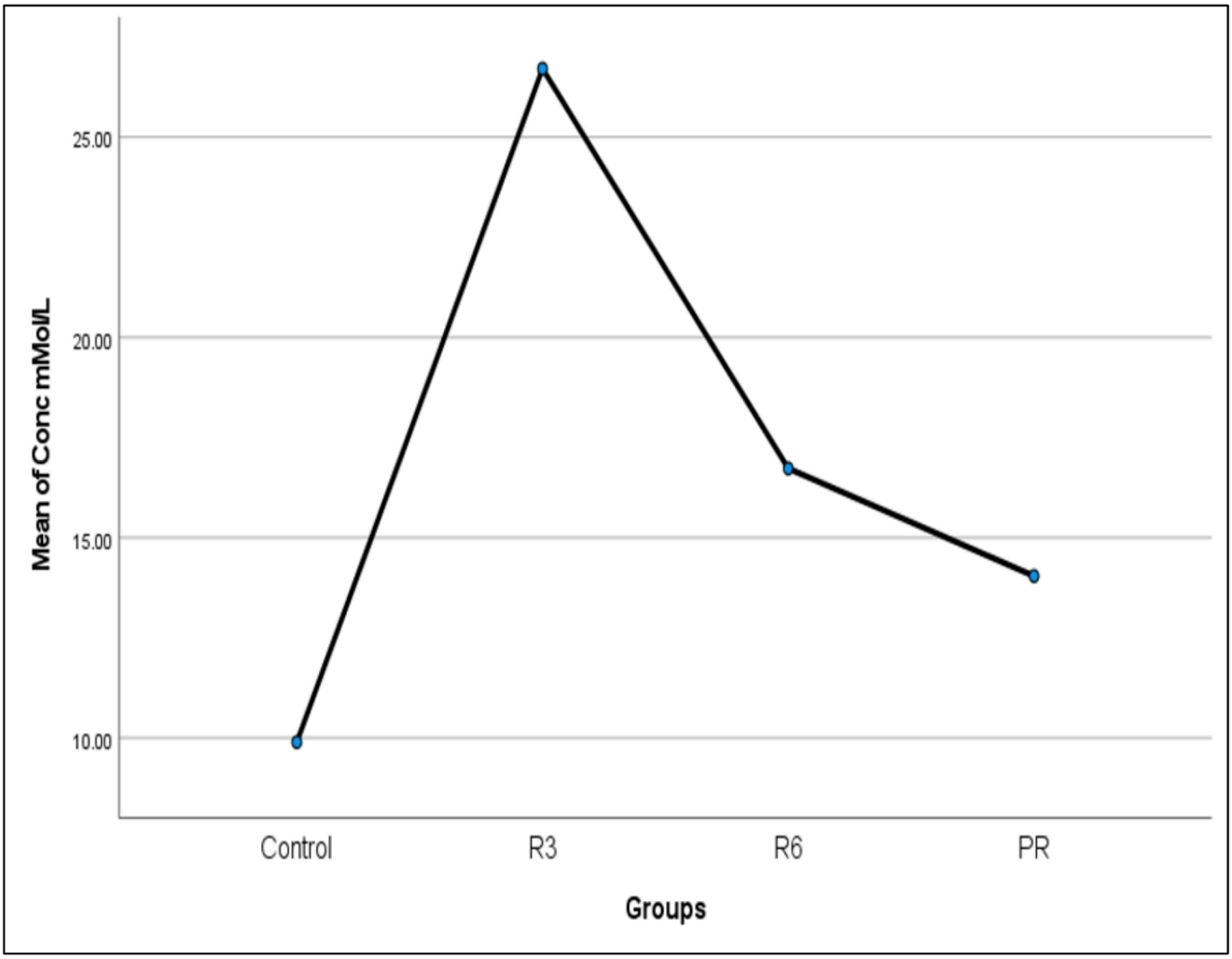


Figure 3

Urea concentration graph