Investigation of circulatory cytokines in patients undergoing intensity-modulated radiotherapy (IMRT) for adenocarcinoma of the prostate and association with acute RT-induced toxicity

A prospective clinical study

Singh, Jagtar; Singh Sohal, Sukhwinder; Ahuja, Kiran; Lim, Aijye; Duncan, Henry; Thachil, Thanuja; De Ieso, Paolo

Published in:
Cytokine

DOI:
10.1016/j.cyto.2020.155108

Published: 01/07/2020

Document Version
Peer reviewed version

Citation for published version (APA):
Investigation of circulatory cytokines in patients undergoing intensity-modulated radiotherapy (IMRT) for adenocarcinoma of the prostate and association with acute RT-induced toxicity: A prospective clinical study

Jagtar Singh1*, Sukhwinder Singh Sohal2, Kiran Ahuja2; Aijye Lim3, Henry Duncan4, Thanuja Thachil5, Paolo De Ieso6

1. College of Health and Human Sciences, Charles Darwin University, Northern Territory, Australia. Email: jagtar.singh@cdu.edu.au
2. School of Health Sciences, University of Tasmania, Tasmania, Australia. Email: sssohal@utas.edu.au; kiran.ahuja@utas.edu.au
3. Department of Anatomical Pathology, Royal Darwin Hospital, Northern Territory, Australia. Email: Aijye.Lim@nt.gov.au
4. Darwin Private Hospital, Royal Darwin Hospital, Northern Territory, Australia. Email: henry.duncan@nt.gov.au
5. Austin Radiation Oncology Centre, Ballarat, Victoria, Australia. Email: thanuja.thachil@bhs.org.au
6. Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia. Email: paolo.deieso@petermac.org

*Corresponding Author

Dr Jagtar Singh
College of Health and Human Sciences,
Charles Darwin University, Northern Territory, Australia.
T. +61 8 8946 4802; F. +61 8 8946 7599; E-mail. cmarkersp534e@gmail.com
ORCID ID. 0000-0002-0457-2650
1. Introduction

Prostate cancer (PC) is the second most common cancer (after lung cancer) in males worldwide, including 1,276,106 new cases and 358,989 deaths in 2018 [1, 2]. The capacity to diagnose and stage of PC is limited and generally based on pre-treatment assessments such as prostate-specific antigen (PSA), TNM stages, and pathologic Gleason score [3]. However, existing pre-treatment assessments cannot be used to predict acute RT-induced toxicity. Therefore, new protein biomarkers are required in RT oncology to improve decision-making, treatment and therapy monitoring for PC patients.

In clinical practice, intensity-modulated radiotherapy (IMRT) is an effective and common treatment modality for locally advanced PC [4]. However, RT has inconsistent success depending on the cancer being treated and it also causes side effects [5]. RT-induced toxicity can be classified as acute or late toxicity. RT-induced toxicity is classified as acute if it occurred during RT and within the first 3 months, they include bowel problem, urinary problem and lethargy [6, 7]. Second malignancy, femoral neck fracture, rectal bleeding, bladder problems and erectile dysfunction are late toxicities which usually occur 3 months after RT completion but it could emerge many months after [6, 8, 9]. In PC, the most common RT-induced toxicity classify as genitourinary (GU) and gastrointestinal (GI) symptoms based on validated scoring criteria [Common Terminology Criteria for Adverse Events (CTC-AE) [10].

Some pro-inflammatory cytokines are believed to play an important role in RT resistance and lead to tumour progression, invasion, and angiogenesis [11-13]. Cytokines are water-soluble, low molecular weight proteins that transport signals between cells [14]. Following RT, researchers believe that normal tissue damage and gene expression changes at the messenger RNA (mRNA) level leads to increased cytokine production within the irradiated area, which then enters the circulation [15, 16]. Ionizing radiation is known to increase the
expression of several cytokines which are involved in inflammation and development of RT-induced toxicity [17]. In several previous studies, cytokines have been examined as novel biomarkers involved in tissue damage response and RT-induced toxicity [10, 15, 18, 19]. Rubin *et al.* were among the first to describe the role of cytokines in RT-induced toxicity [20]. Cytokines as biomarkers have gained much attention in the last few years, with the intention of the screen and notify the degree and severity of toxicity from RT.

The goals of this study were to assess the levels of cytokines in blood plasma before and after RT and interrogate any association with acute GU and GI toxicity respectively.

2. **Material and methods**

2.1 **Study population and patient information**

Eighteen patients were recruited who had agreed to undergo curative external beam RT (EBRT) +/- ADT for intermediate to high-risk PC using the D’Amico classification [21]. All patients were diagnosed between 2nd July 2015 and 21st April 2016 at Royal Darwin Hospital (RDH). Eligible patients were ≥18 years old, had histologically confirmed prostate adenocarcinoma, and had an ECOG (Eastern Cooperative Oncology Group) performance status of 0 to 1 and no history of prior surgery to the prostate. Exclusion criteria include prior prostate EBRT or brachytherapy, metastatic disease at presentation, prior history of malignancy (excluding non-melanoma skin cancer), and serious medical or psychiatric illness precluding safe administration of RT. Using the D’Amico classification, selected patients were classified into the following three groups: low risk, ≤ cT2a, PSA <10 ng/ml and GS ≤ 6; intermediate-risk, cT2b, PSA 10–20 ng/ml, GS = 7; and high risk, ≥ cT2c, PSA > 20 ng/ml, GS ≥ 9 – 10 [21].
2.2 Radiation therapy

All patients had fiducial markers inserted prior to RT and had daily cone beam imaging during treatment. Treatment planning was based on computed tomography (CT) performed with empty rectum, comfortably filled bladder, with patients in the prone position. Clinical target volume (CTV) included the prostate gland or the prostate gland plus seminal vesicles. A 1cm margin was added around the CTV to define the planning target volume (PTV), expect the boundary between the anterior rectal wall and the prostate, where a 0.7 cm margin was used.

2.3 Grading RT-induced acute toxicity

Patients were asked to complete questionnaires concerning symptoms of bladder or rectal dysfunction prior to RT, on the last week of RT and 3 months after the completion of RT as part of their standard care. International Index Prostate Symptom Scoresheet (IIPS) [22, 23] and Expanded Prostate Cancer Index Composite (EPIC) [24] were used to interrogate the acute bladder and rectal toxicities respectively. The total score can range from 0 to 35 for IIPS and 0 to 28 for EPIC respectively (asymptomatic to very symptomatic). The RT-induced toxicity was considered as mild if the total score is equal or less than 7, while it was considered as moderate if total score range was 8-19, and it was considered as severe if the total score range is 20-35 [23, 25].

2.4 Blood sampling and plasma processing

Patients’ blood was taken before immediately preceding the initiation of RT, at the end of RT and 3 months from the completion of RT. At each sampling, 5-10 ml of peripheral blood was drawn into blindly coded 5-7 ml vacutainer tubes containing powdered lithium heparin (14 Units/ml blood). Blood samples were immediately placed on ice for transport to the
laboratory, aliquoted into conical 15 ml tubes and centrifuged (3000 \times g \times 20 \text{ min}) to separate out the plasma. The platelet-free plasma layer was separated from the blood, transferred into coded cryotubes and frozen at –80°C until they were analysed. The analysis was carried out under blind conditions and in accordance with the guidelines established by the Charles Darwin University committee for handling biohazardous material.

2.5 Assessment of blood plasma cytokines

The expression levels of cytokines were measured using sandwich enzyme-linked immunosorbent assays (ELISA) which are commercially available in kits from Life Technologies Australia Pty Ltd. The cytokine concentration (colour) was quantified using a Titertek Multiskan MCC/340 plate reader at the appropriate wavelength dictated by the particular kit utilized. Each assay was run against a standard curve with a full range predetermined for each cytokine and sample source. These kits were designed to detect cytokines levels using a target-specific antibody on pre-coated 96 well microplates.

2.6 Statistical analysis

Statistical analysis was performed using GraphPad Prism 7. The Mann-Whitney U test was used to compare cytokine levels for pre-RT versus post-RT. The Wilcoxon sign-rank test was used to determine the effect of RT on cytokine levels using aggregated median cytokine levels at before and after IMRT. Spearman’s correlation test was used to determine the correlation between cytokine levels and patient-reported GU and GI toxicity, respectively. For all analyses, p \leq 0.05 was taken as significant.
3. Results

The clinical characteristics of patients in this study are summarised in table 1. The mean age of PC patients at the time of diagnosis was 66.8 years (range, 53 - 80 years). The mean pre-treatment serum PSA level was 16.03 ng/ml (range, 4 – 71 ng/ml) and post-RT serum PSA were 0.34 ng/ml (range, 0.05 – 3.48 ng/ml). The mean GS was 8 (range, 6 – 10). Four (23%) patients had a GS of 3 + 4 = 7, five (28 %) patients had GS of 4 + 3 = 7, three (16.5 %) patients had GS of 4 + 4 = 8, three (16.5 %) patients had GS of 4 + 5 = 9, one (5 %) patient had GS of 5 + 4 = 9 and two (11 %) patients had GS of 5 + 5 = 10. Patients were planned and treated using IMRT. Definitive IMRT patients received a total dose of 78 Gy in 39 fractions (n = 4) and 80 Gy in 40 fractions (n = 14).
**Table 1: Clinicopathological characteristics of patient population**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number of patients</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>66.83 ± 7.93 (53 – 80)</td>
<td></td>
</tr>
<tr>
<td>cT-stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• T1c</td>
<td>1</td>
<td>5%</td>
</tr>
<tr>
<td>• T2a</td>
<td>3</td>
<td>16.5%</td>
</tr>
<tr>
<td>• T2b</td>
<td>4</td>
<td>23%</td>
</tr>
<tr>
<td>• T2c</td>
<td>2</td>
<td>11%</td>
</tr>
<tr>
<td>• T3a</td>
<td>3</td>
<td>16.5%</td>
</tr>
<tr>
<td>• T3b</td>
<td>5</td>
<td>28%</td>
</tr>
<tr>
<td>Gleason score</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• 3 + 3 = 6</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>• 3 + 4 = 7</td>
<td>4</td>
<td>23%</td>
</tr>
<tr>
<td>• 4 + 3 = 7</td>
<td>5</td>
<td>28%</td>
</tr>
<tr>
<td>• 4 + 4 = 8</td>
<td>3</td>
<td>16.5%</td>
</tr>
<tr>
<td>• 4 + 5 = 9</td>
<td>3</td>
<td>16.5%</td>
</tr>
<tr>
<td>• 5 + 4 = 9</td>
<td>1</td>
<td>5%</td>
</tr>
<tr>
<td>• 5 + 5 = 10</td>
<td>2</td>
<td>11%</td>
</tr>
<tr>
<td>Pre-RT PSA (ng/ml)</td>
<td>16.03 ± 15.81 (range, 4 – 71)</td>
<td></td>
</tr>
<tr>
<td>Post-RT PSA (ng/ml)</td>
<td>0.35 ± 0.77 (range, 0.05 – 3.48)</td>
<td></td>
</tr>
<tr>
<td>Radiation dose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• 78 Gy in 39 fractions</td>
<td>4</td>
<td>23%</td>
</tr>
<tr>
<td>• 80 Gy in 40 fractions</td>
<td>14</td>
<td>77%</td>
</tr>
</tbody>
</table>

Values for age are presented as the mean ± standard deviation; cT-stage, clinical tumour stage; RT, radiotherapy; PSA, prostate-specific antigen and Gy, gray, unit of radiation dose.
3.1 Baseline (pre-RT) versus post-RT cytokines levels

The concentration levels of anti-inflammatory transforming growth factor-β1 (TGF-β1) were elevated (mean ±SD, 765.2 ± 426.3 to 915.9 ± 539.2) at the end of RT when compared with pre-RT cytokines levels. Though the levels of pro-inflammatory interleukin-6 (IL-6) and IL-8 (mean ±SD, 10.6 ± 3.2 to 16.2 ± 15.5 and 1.2 ± 0.1 to 1.3 ± 0.1) were found to be altered 3 months post-RT completion, only IL-8 levels were found to be statistically significant (p = 0.05).

3.2 Correlation between plasma cytokine levels and patient-scored RT-induced toxicity

We also sought to determine if there was an association between cytokine levels and patient-reported acute GU and GI toxicity graded prospectively (summarized in Table 2 and 3). Levels of pro-inflammatory tumour necrosis factor-α (TNF-α) (mean ±SD, 13.8 ± 1.9 to 17.7 ± 3.7 and 15.4 ± 4.2 to 23.9) and IL-6 (mean ±SD, 9.8 ± 3.6 to 12.1 ± 4.5 and 18.7 ± 20.3 to 44.6) increased as the severity of GU toxicity increased at post-RT and after 3 months completion of RT. However, it did not show statistical significance difference. In contrast, levels of anti-inflammatory TGF-β1 decreased (mean ±SD, 1201.7 ± 892.9 to 726.5 ± 657.2 and 936.0 ± 580.3 to 343.1, respectively) as the severity of GU toxicity increased at the end of RT and 3 months post-RT completion. Again, we could not find a significant difference.
Table 2: Expression of cytokines along the severity of acute GU RT-induced toxicity

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Phase of Blood</th>
<th>GU toxicity = 0</th>
<th>GU toxicity = 1</th>
<th>GU toxicity = 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>End of RT</td>
<td>5</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>3 months after RT</td>
<td>8</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>TNF-α</td>
<td>End of RT</td>
<td>13.8 ± 1.9 (pg/ml)</td>
<td>18.6 ± 3.6 (pg/ml)</td>
<td>17.7 ± 3.7 (pg/ml)</td>
</tr>
<tr>
<td></td>
<td>3 months after RT</td>
<td>15.4 ± 4.2 (pg/ml)</td>
<td>17.1 ± 3.9 (pg/ml)</td>
<td>23.9 (pg/ml)</td>
</tr>
<tr>
<td>TGF-β1</td>
<td>End of RT</td>
<td>1201.7 ± 892.9 (pg/ml)</td>
<td>858.3 ± 256.4 (pg/ml)</td>
<td>726.5 ± 657.2 (pg/ml)</td>
</tr>
<tr>
<td></td>
<td>3 months after RT</td>
<td>936.0 ± 580.3 (pg/ml)</td>
<td>610.1 ± 254.9 (pg/ml)</td>
<td>343.1 (pg/ml)</td>
</tr>
<tr>
<td>IL-6</td>
<td>End of RT</td>
<td>9.8 ± 3.6 (pg/ml)</td>
<td>10.1 ± 1.9 (pg/ml)</td>
<td>12.1 ± 4.5 (pg/ml)</td>
</tr>
<tr>
<td></td>
<td>3 months after RT</td>
<td>18.7 ± 20.3 (pg/ml)</td>
<td>10.2 ± 2.6 (pg/ml)</td>
<td>44.6 (pg/ml)</td>
</tr>
<tr>
<td>IL-8</td>
<td>End of RT</td>
<td>1.1 ± 0.1 (pg/ml)</td>
<td>1.2 ± 0.1 (pg/ml)</td>
<td>1.2 ± 0.1 (pg/ml)</td>
</tr>
<tr>
<td></td>
<td>3 months after RT</td>
<td>1.3 ± 0.1 (pg/ml)</td>
<td>1.2 ± 0.1 (pg/ml)</td>
<td>1.2 (pg/ml)</td>
</tr>
</tbody>
</table>

Values for age are presented as the mean ± standard deviation; TNF-α, Tumour necrosis factor-alpha; TGF-β1, Tumour growth factor-beta1; IL-6, Interleukin-6; IL-8, Interleukin-8; GU toxicity (0 = mild, 1 = moderate and 2 = severe toxicity).
Table 3: Expression of cytokines along the severity of acute GI RT-induced toxicity

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Phase</th>
<th>GI toxicity = 0</th>
<th>GI toxicity = 1</th>
<th>GI toxicity = 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>End of RT</td>
<td>13</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>3 months after RT</td>
<td>9</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td><strong>TNF-α</strong></td>
<td>End of RT</td>
<td>16.9 ± 3.8 (pg/ml)</td>
<td>15.4 ± 3.3 (pg/ml)</td>
<td>19.3 ± 3.6 (pg/ml)</td>
</tr>
<tr>
<td></td>
<td>3 months after RT</td>
<td>14.9 ± 4.0 (pg/ml)</td>
<td>17.9 ± 3.7 (pg/ml)</td>
<td>23.9 (pg/ml)</td>
</tr>
<tr>
<td><strong>TGF-β1</strong></td>
<td>End of RT</td>
<td>972.7 ± 536.5 (pg/ml)</td>
<td>362.5 ± 40.3 (pg/ml)</td>
<td>269.3 ± 10.4 (pg/ml)</td>
</tr>
<tr>
<td></td>
<td>3 months after RT</td>
<td>882.1 ± 558.5 (pg/ml)</td>
<td>627.6 ± 250.5 (pg/ml)</td>
<td>343.1 (pg/ml)</td>
</tr>
<tr>
<td><strong>IL-6</strong></td>
<td>End of RT</td>
<td>9.9 ± 2.5 (pg/ml)</td>
<td>8.7 ± 1.7 (pg/ml)</td>
<td>14.3 ± 4.6 (pg/ml)</td>
</tr>
<tr>
<td></td>
<td>3 months after RT</td>
<td>17.7 ± 18.9 (pg/ml)</td>
<td>10.2 ± 2.8 (pg/ml)</td>
<td>44.6 (pg/ml)</td>
</tr>
<tr>
<td><strong>IL-8</strong></td>
<td>End of RT</td>
<td>1.2 ± 0.1 (pg/ml)</td>
<td>1.3 ± 0.1 (pg/ml)</td>
<td>1.2 ± 0.1 (pg/ml)</td>
</tr>
<tr>
<td></td>
<td>3 months after RT</td>
<td>1.3 ± 0.1 (pg/ml)</td>
<td>1.2 ± 0.1 (pg/ml)</td>
<td>1.2 (pg/ml)</td>
</tr>
</tbody>
</table>

Values for age are presented as the mean ± standard deviation; TNF-α, Tumour necrosis factor-alpha; TGF-β1, Tumour growth factor-beta1; IL-6, Interleukin-6; IL-8, Interleukin-8; GI toxicity (0 = mild, 1 = moderate and 2 = severe toxicity).
Moreover, levels of pro-inflammatory TNF-α (mean ±SD, 16.9 ± 3.8 to 19.3 ± 3.6 and 14.9 ± 4.0 to 23.9, respectively) and IL-6 (mean ±SD, 9.9 ± 2.5 to 14.3 ± 4.6 and 17.7 ± 18.9 to 44.6, respectively) increased as the severity of GI toxicity increased at post-RT and 3 months post-RT completion. However, concentration levels of pro-inflammatory TNF-α and IL-6 were not statistically different when compared with the severity of GI toxicity. On the other hand, levels of anti-inflammatory TGF-β1 (mean ±SD, 972.7 ± 536.5 to 269.3 ± 10.4 and 882.1 ± 558.5 to 343.1, respectively) decreased as the severity of GI toxicity increased at the end of RT and after 3 months completion of RT. Though, this difference was not statistically significant, maybe due to the limited sample size. In figures 1 and 2 maximum levels of cytokines were plotted against the maximum patient-scored acute GU and GI toxicity at the end of RT and after 3 months of RT completion.
Figure 1: Plasma cytokines levels against acute RT-induced toxicity at the end of RT. Maximum expression of (TNF-α, IL-6) as a function of maximum toxicity. However, TGF-β1 is decreasing while increasing toxicity. Closed circles, GU toxicity; open circles, GI toxicity.
Figure 2: Plasma cytokines levels against acute RT-induced toxicity after 3 months of RT completion. Maximum expression of (TNF-α, IL-6) as a function of maximum toxicity. Though, TGF-β1 is decreasing while increasing toxicity. Closed circles, GU toxicity; open circles, GI toxicity.
4. Discussion

During planning RT for the treatment of PC, radiation oncologists consider several tumour and treatment-related factors. These factors comprise patterns of regional tumour spread, to confirm coverage of local tumour extension, uncertainties in positioning the patient and tumour and organ movement during and between treatments. To accomplish these goals, normal tissues surrounding the tumour are irradiated, which may result in symptomatic injury. These injuries are acute or chronic and have an influence on the quality of life of PC patients.

RT is a well-established treatment for PC in organ-confined disease, including early and locally advanced PC [26, 27]. Most studies investigating an association between cytokine levels and RT-induced toxicity have concentrated on cytokine measurements prior to and during RT treatment [10]. Several previous studies reported that levels of cytokines such as IL-1, TGF-β, and TNF-α, IL-6, and IL-4 were increased during or after RT completion [20, 28, 29]. Johnke et al. also revealed that concentration levels of TGF-β, IL-1β, and IL-6 were increased at the end of RT [30]. Further clinical studies also identified that expression levels of cytokines such as TGF-β1 and TGF-β2, TNF-α and IL6 were elevated during IMRT in the blood patients with PC [10, 31, 32]. In our clinical study, levels of anti-inflammatory TGF-β1 were increased in post-RT blood plasma; however, those changes were not statically significant. Although levels of pro-inflammatory IL6 and IL8 were found to be altered 3 months post-RT completion, only changes in IL-8 levels were found to be statistically significant.

The importance of dose escalation for tumour control has been revealed in many randomised trials [33, 34]. By increasing the RT dose, the risk of developing complications caused by injury to the bladder, prostatic urethra and rectum also increase [35]. The possible association between RT dose and the effects of RT in PC has been observed by several
investigators [7, 8]. The release of intracellular molecules from injured cells by RT triggers
the up-regulation of the pro-inflammatory IL-1β and TNF-α cytokines [36]. Christensen et al.
reported that levels of IL-2 and IL-1 were elevated during RT over Pre-RT and significantly
associated with increased GU toxicity [10]. A significant association between levels of TGF-
β1 and development of RT-induced fibrosis was also observed in breast cancer patients [37].
Furthermore, levels of TGF-β1 were elevated in patients during RT, who developed RT-
induced lung toxicity (RILT) [38]. This clinical study also agrees with other clinical studies
that elevated cytokine expression post-RT was associated with RT-induced lung toxicity [39-
42]. In our clinical study, levels of pro-inflammatory TNF-α and IL-6 increased after RT and
they were associated with the higher probability for GU toxicity. However, the level of anti-
inflammatory TGF-β1 was found to be inversely proportional to GU toxicity in our study in
contrast to previous studies. However, we did not find any significance difference.

RT treatment for PC also exposes a portion of the lower GI tract to ionizing radiation and
therefore carries a risk of GI toxicity. Modifications of intestinal motility and peristalsis such
as high stool frequency, loose stools and rectal urgency can greatly affect the quality of life of
PC patients [43]. A previous study reported that increased level of pro-inflammatory IL-6
was associated with a higher probability for acute GI toxicity; however, it did not find any
significant difference [10]. Moreover, levels of pro-inflammatory TNF-α were elevated in
patients who had GI symptoms [29]. Another study also reported that an elevated level of
pro-inflammatory IL-1 was associated with an increased probability of GI toxicity [44]. In the
current study, levels of pro-inflammatory TNF-α and IL-6 were also increased and associated
with a higher probability for acute GI toxicity. On the other hand, again level of anti-
inflammatory TGF-β1 was found to be inversely proportional to GI toxicity in our study in
sharp contrast to the former studies. However, there was no statistically significant
association between cytokine levels and severity of acute GI toxicity.
The clinical study reported here is proposed to be investigative and to guide future larger trials. We observed that levels of pro-inflammatory cytokines TNF-α and IL-6 were increased and anti-inflammatory TGF-β1 decreased after prostate IMRT. Noticeably, we have found a promising association between altered levels of cytokines and acute GU and GI toxicity, although our results are not statistically significant, likely due to limited sample size. Further studies are clearly needed to attribute the altered (increased/decreased) levels of pro-inflammatory TNF-α, IL-6 and anti-inflammatory TGF-β1 cytokines in PC patients as a biomarker of malignancy and RT-induced toxicity.

5. Conclusion

In this clinical observation study, a trend towards increased pro-inflammatory cytokine levels with increased acute GU and GI toxicity was observed, though the difference was not statistically significant, likely due to limited sample size. Further studies with larger cohorts are needed, especially to establish the relationship between levels of pro-inflammatory TNF-α, IL-6 and anti-inflammatory TGF-β1 cytokines and acute RT-induced toxicity.

Declarations

Ethics approval and consent to participate

All participants were informed of the tentative nature of this observation clinical study and signed informed consent forms prior to their inclusion in this study. This clinical study has been approved by the Human Research and Ethics Committee (HREC: 2015-2385) of the Northern Territory (NT) and the Department of Health and Menzies School of Health Research. The research team collected previous prostate tissue biopsies, blood samples at different time points during RT treatment. Medical and pathology records from Royal Darwin
Hospital (RDH) and Alan Walker Cancer Care Centre (AWCCC) were interviewed during this study.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Conception and design: JS, SSS, TT and PDI
Clinical data and samples collection: JS, HD, AL, TT and PDI
Analysis of experimental data: JS, SSS and KA
Drafting of the article or critical revision: All authors
Final approval of the article: All authors

Acknowledgements

This clinical study was supported by College of Health and Human Sciences, Charles Darwin University, Australia. The authors would also like to acknowledge AWCCC, RT technicians for their support in blood sampling and clinical data collection for this study, and the patients who gave their time so freely to participate in this study.
References


[18] T.L. McDonald, A.Y. Hung, C.R. Thomas, et al., Localized External Beam Radiation Therapy (EBRT) to the Pelvis Induces Systemic IL-1Beta and TNF-Alpha Production:
Role of the TNF-Alpha Signaling in EBRT-Induced Fatigue, Radiat. Res. 185 (2016) 4-12.


