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Salivary Lactate Dehydrogenase as a Predictive Marker for Radiation-induced Dermatitis in Head and Neck Cancers: A Preliminary Study

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Abstract

Background: Radiation dermatitis is known to be a major side-effect occurring following cancer treatment. We conducted the present study to understand whether salivary lactate dehydrogenase (LDH) could be conducive to predict the development of radiation-dermatitis in the head and neck cancer (HNC) patients undergoing curative radiotherapy (60-70 Gy).

Method: This was a prospective study performed on HNC patients requiring curative radiotherapy. Saliva was collected at two points from the willing volunteers. The first time point was prior to the first fraction of 2 Gy radiation and the second one was 24 hours after the first fraction and before exposure to the second fraction. The saliva collected at the both time points were analyzed for the levels of salivary LDH using standard procedure. The patients were provided with the standard care throughout the treatment period and the incidence and severity of radiation dermatitis was noted down using a proforma sheet throughout the 7-week treatment period.

Results: The results suggested that with exposure to 2 Gy fraction, there was an increase in the level of salivary LDH (387.11 ± 18.98 IU/L vs. 368. 13 ± 19.56IU/L); this increase was significant (t = 20.06 and $P < 0.001$). The LDH data was stratified based on the severity of dermatitis [mild (grades 1 and 2) vs. severe (grades 3 and 4)] in accordance to the Radiation Therapy Oncology Group/European Organization for Research and Treatment Cancer (RTOG) grading. The LDH values were subjected to Karl Pearson's correlation analysis with the grade of dermatitis and the results indicated a P value of 0.019 and R value of 0.24.

Conclusion: For the first time, our study revealed that salivary LDH could be a useful marker to understand the development of radiation-induced dermatitis in HNC patients undergoing curative radiotherapy. The most advantageous aspect herewith is that the collection of saliva does not require skilled people or special equipment; it could be done at repeated intervals and without causing any invasive process.

Keywords: Head and neck neoplasms, Saliva, Lactate dehydrogenase, Radiotherapy, Radiodermatitis

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Introduction

In the treatment of cancer growing in the head and neck (H&N), external beam radiation therapy is an important modality. Estimations suggest that it is used in nearly 60% of patients with curative intent.[1, 2] In clinics, depending on the general health of the individual and the tumor location and characteristics, radiation may be used either alone or in combination with very low doses of anticancer drug cisplatin to ensue better tumor regression and control. However, in some cases, the use of curative radiotherapy to the H&N region is followed by severe side-effects, like mucositis, dermatitis, dysgeusia salivary dysfunction, and hematological toxicities; they lower the quality of life and do not result in the expected therapeutic benefits.[1,2]

Clinically, the incidence of radiation-induced dermatitis is a highly prevalent side-effect of ionizing radiation. Realistic appraisal suggests that almost 85% of all the patients undergoing radiotherapy to the HNC region develop moderate-to-severe skin reaction when the dose exceeds 30-40 Gy.[3] Dermatitis varies in terms of severity from moderate to severe erythema and moist scaling. The symptoms may include skin dryness, itching discomfort, and pain.[3] When severe radiodermatitis is fiercely painful, the specialist might have to opt for treatment breaks or, in the worst-case scenario, reduce the planned treatment dose.[3,4] Conventionally, in clinics, the use of dermaprotective topical agents is largely anecdotal and conventional derma care products, containing steroidal, non-steroidal, and metallic topical preparations and dressings, are used.[4]

Research in the field of diagnostics has suggested that biochemical endpoint from the site of the treatment and/or body fluid is always useful.[5] Lactate dehydrogenase (LDH) (EC1.1.1.27; L-lactate: nicotinamide adenine dinucleotide [NAD+] oxidoreductase) is of particular importance in the glycolytic pathway.[6] It belongs to the 2-hydroxyacid oxidoreductase family and is involved in catalyzing the reversible conversion of pyruvate and lactate during glycolysis and gluconeogenesis.[6] From a clinical perspective, LDH is one of the well-studied enzymes that is clinically utilized as an important marker in cardiology, hematology, hepatology, and oncology.[7]

Ionizing radiation is a potent cytotoxic agent and studies with cultured mammalian cells have shown that on exposure, the levels of LDH increases in the cultured media;[8,9] the latter has been observed in the serum of exposed laboratory animals,[10-12] and in humans undergoing curative radiotherapy for their cancer.[13,14] Recent reports have also suggested that the levels of salivary LDH rose in HNC patients undergoing curative radiotherapy and that the levels are correlated with the radiation-induced mucositis.[15]

In the treatment of HNC, the treatment planning is focused mainly on the tumor cell kill. However, the skin at the site of radiation also gets exposed to a fraction of the planned dose, and depending on the incident skin dose and the inherent radiosensitivity of the patient dermatitis will manifest as mild to severe grade. Physiologically, LDH is released when cells are exposed to cytotoxic agent and its level in serum is an important prognostic factor for many diseases.[7] When buccal cells are exposed to the cytotoxic effects of radiation, LDH is released in to the saliva in the oral cavity.[15] As both skin cells and buccal cells incur the radiation damage during the course of the curative radiotherapy, we hypothesized that the level of salivary LDH could be a marker for dermatitis. Considering these facts, the current study was planned and carried out to ascertain the value of salivary LDH as a marker to indicate severity of radiodermatitis in HNC patients undergoing curative radiotherapy.

Materials and Methods

We conducted this prospective study from October 2012 to September 2015 in the Departments of Radiation Oncology and Biochemistry at Father Muller Medical College Hospital, Mangalore, India. The subjects comprised histopathologically confirmed adult H&N cancer patients scheduled to receive the standard curative radiotherapy (60-70 Gy) as described earlier.[15] The inclusion criteria included patients newly diagnosed with HNC, not having

received radiotherapy/chemo-irradiation or chemotherapy for the cancer and above the age of 18 years. While the exclusion criteria included patients with metastatic cancer, patients who had received chemotherapy or radiation treatment previously, patients using high doses of non-steroidal anti-inflammatory drugs, patients with co-morbid conditions like poorly controlled diabetes mellitus, hypertension; patients with mental illness like schizophrenia, bipolar disorders; women patients who were pregnant and patients who are not willing to be a part of the study. The Institutional Ethical Committee (FMMC/FMIEC/877/2012) approved the study and the research was conducted as per the ethical principles specified by the Declaration of Helsinki for research with humans.

Radiation treatment

The participants herein were scheduled to receive external irradiation at an average energy level of 6 Me V from a linear accelerator (Varian Medical systems, Unique 2012, Palo Alto, CA, USA). The patients were planned to receive a curative target dose of 60-70 Gy five days a week without any intended gap and no more than one fraction per day of 2 Gy for 6-7 consecutive weeks. They received cisplatin infusion (50 mg/m2/day IV) as per standard guidelines.[16,17]

Saliva collection

During the first visit, one of the student investigators (PS, FK or TG) introduced the nature and purpose of the study to the eligible patients meeting the inclusion criteria, in either English or their mother tongue (Kannada, Tulu, or Malayalam). The subjects were informed that they had the right to withdraw from the study at any time and that their non-willingness to participate will not stop the necessary planned treatment for them. The willing patients were then included in the study and a written informed consent was collected by one of the authors (PS, FK, or TG).

Unstimulated saliva was collected in accordance with the method suggested by Navazesh,[18] and at two time points only: (1) before the start of radiation treatment (day 1;

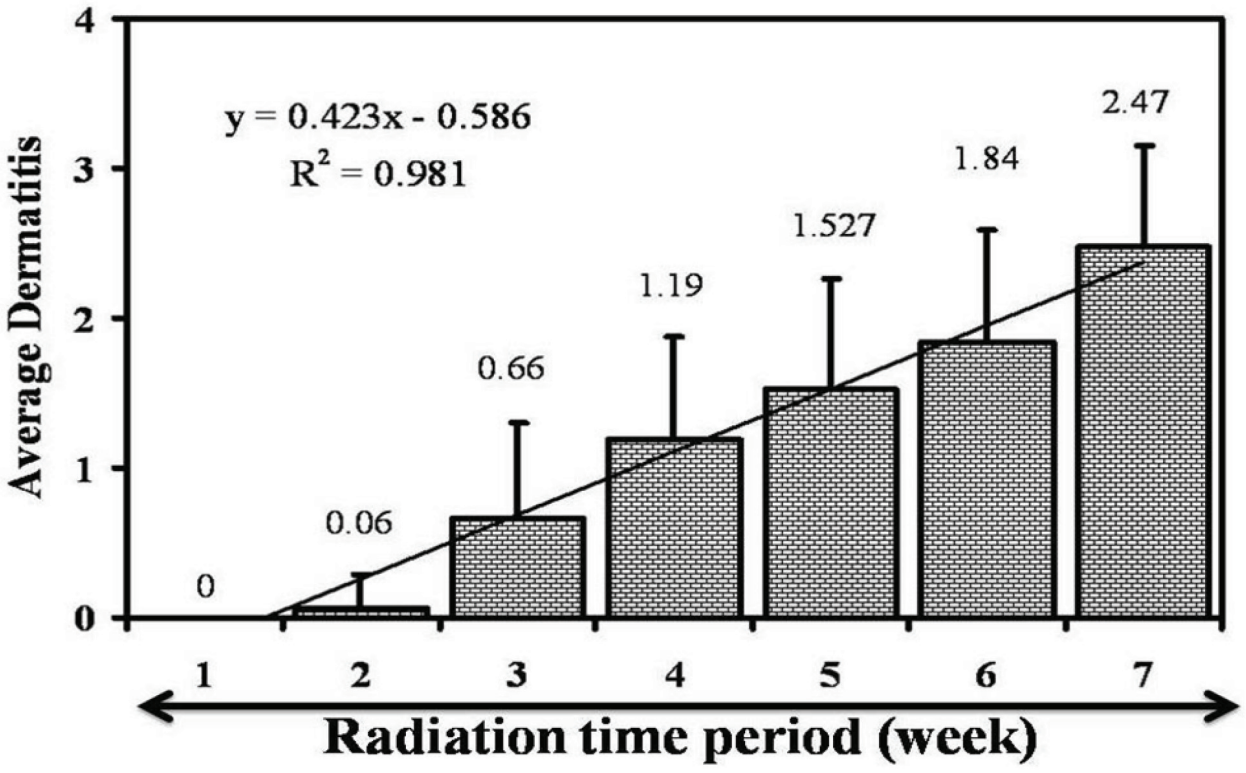

Figure 1. This figure shows the average mucositis during the 7-week treatment course for head and neck cancer patients treated with radiation (60-70 Gray).

before exposure to the first fraction of 2 Gy) and (2) the next day (day 2; after 24 h of the first fraction and before exposure to the second fraction of 2 Gy). Every subject was asked to rinse the mouth with distilled water thoroughly so that any food debris would be removed. Subsequently, after 10 minutes, they were requested to salivate into a sterile plastic. Salivary flow rate (ml/min) was measured via the following formula.[18]

$$= \frac{\text{Weight of the container with Saliva (g) Weight of the container without saliva (g)}}{\text{Duration of saliva collection}}$$

Once saliva was collected in the plastic container, it was immediately transported to the biochemistry laboratory in an ice box. The collected saliva was centrifuged at 3000 rpm for 10 minutes, and the supernatants were stored in a cold refrigerator (-20ºC).

Estimation of LDH in saliva

The stored saliva was removed from the cold refrigerator, thawed, and analyzed using appropriate blanks, controls, and standards using the UV-visible spectrophotometer (Shimadzu, Japan). We performed the LDH assay with the kinetic spectrophotometric method described by Demetriou et al.;[19] it was done using the reagent kit of Roche diagnostics in accordance with the protocol of the user manual provided by the manufacturers using the suitable controls. The assay is based on LDH-catalyzed reduction of pyruvate with NADH to form NAD+. The rate of oxidation of NADH to NAD+ was measured as the decrease in absorbance at 340 nm and expressed in terms of Units/L.

Skin care and clinical evaluation for dermatitis

The patients were advised to use lukewarm water to wash and with gentle detergent, not to use hair shampoo or razor blade, not to rub or scratch irradiated skin, to use a hat or umbrella for covering skin from direct sunlight, to pat skin dry with a soft towel after washing, and to keep irradiated skin dry. The assessment was undertaken on a weekly basis on Fridays based on the criteria of the Radiation Therapy Oncology Group/European Organization for Research and Treatment Cancer.[20] In every examination, one of the investigators (SP) considered the score for the worst toxicity in the treatment field as

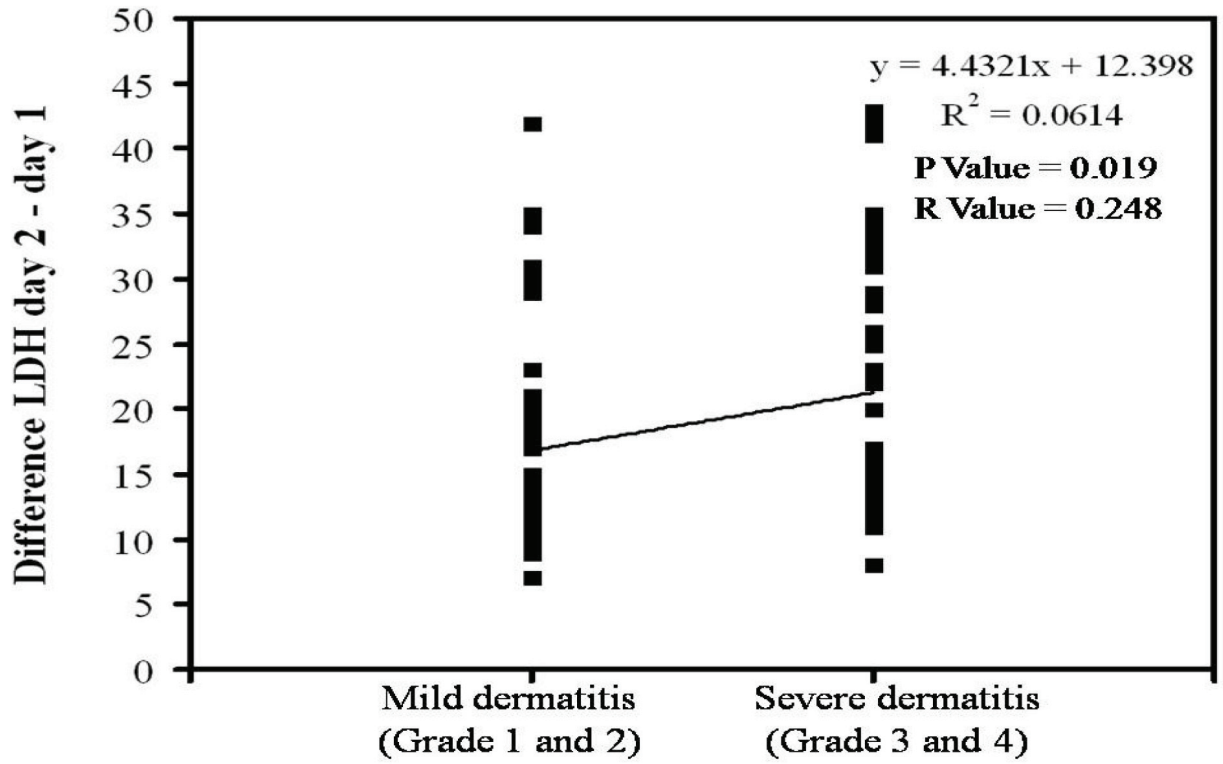

Figure 2. This figure shows the association between the degrees of radiodermatitis and the difference in the salivary lactate dehydrogenase (LDH) values (obtained by deduction of the value of day 2 from day 1).

Table 1. Patients' age, gender, and habits details

Parameter	Groups	HNC (N= 89)	Percentage (%)
	Mean	**53.90 ± 10.2**	
Age	Below 30	2	2.25
	31 to 40	6	6.74
	41 to 50	27	30.34
	51 to 60	37	41.57
	61 to 70	11	12.36
	Above 70	6	6.74
Sex	Male	71	79.78
	Female	18	20.22
Cigarette smoking	Yes	60	67.42
	No	29	32.58
Drinking alcoholic beverages	Yes	73	82.02
	No	16	17.98
Chewing tobacco	Yes	46	51.69
	No	43	48.31
Snuff inhaling	Yes	22	24.72
	No	67	75.28

HNC: Head and neck cancer

described earlier.[20-22] All the patients were provided with the standard medical care.

Statistical analysis

The values were expressed as mean with standard deviation. Statistical significance for the difference concerning LDH was carried out using the paired "t-test". The correlation among the differences between the salivary LDH (day 2 and day 1) and the severity of radiation dermatitis score [mild verses severe] was analyzed with Karl Pearson's correlation analysis. Statistical analyses were performed with SPSS software (SPSS Inc., Chicago, IL) and a value of $P < 0.05$ was considered to be significant.

Results

A total of 89 evaluable cases could be collected with the salivary LDH assay results and dermatitis scores with complete data for the radiation treatment in a 7-week period. Out of these subjects, 71 were male and 18 were female. Their mean age was 53.90 ± 10.20 years with 41.57% being in the age range of 51 to 60 (Table 1). Regarding their habits, it was observed that 67.42%, 82.02%, 51.69%, and 24.72% practiced tobacco smoking, alcohol consumption, snuff chewing, and snuff inhalation, respectively (Table 1). In the study, majority of the patients were affected with cancer in the tongue (32.58%) followed by buccal mucosa (15.73%) and oropharynx (8.99%) (Table 2). The histopathology details ascertained in accordance to the TNM classification suggested that 47.19% of the patients had the tumor stage of T2 stage (Table 2). The data on the regional nodes suggested that majority (50.56%) had N2 regional lymph node spread status (Table 2). The other important observation was that 84.27% of the patients had M0 (cancer has not spread to other parts of the body), while 15.73% had Mx (metastasis cannot be measured.) status for distant metastasis evaluation (Table 2).

Exposure to radiation increased the degree of dermatitis throughout the 7-week period of the study; figure 1 represents the findings in this regard. The first case of dermatitis was observed on day 13 and by day 47, all the patients had developed grade 1 dermatitis. With the increase in the dose of exposure, there was an exponential rise in the number of patients with grade 2 dermatitis and a total of 96.63% (86/89) developed it by the end of the radiation treatment. Grade 3 dermatitis was initially seen at the end of week 4 and by the end of the treatment, 47.19% (42/89) had developed it. The most severe and intolerable grade 4 dermatitis developed only in 5.62% (5/89) of the patients and in the last week (week 7) of the treatment.

The results of the salivary LDH indicated an

Table 2. Tumor characteristics of the volunteers who took part in the study

	Frequency (N = 89)	Percentage (%)
Cancer site		
Alveolus	2	2.25
Buccal mucosa	14	15.73
Floor of the mouth	3	3.37
Gingivobuccal sulcus	4	4.49
Hypopharynx	7	7.87
Larynx	1	1.12
Maxilla	1	1.12
Oropharynx	8	8.99
Parotid	1	1.12
Post cricoid	1	1.12
Posterior pharyngeal wall	1	1.12
Pyriform sinus	3	3.37
Retromolartrigone	2	2.25
Submandible	1	1.12
Supraglottis	4	4.49
Tongue/base of tongue	29	32.58
Tonsil	5	5.62
Vallecula	3	3.37
Primary (T)		
T1	4	4.49
T2	42	47.19
T3	32	35.96
T4	11	12.36
Regional nodes (N)		
N0	22	24.72
N1	17	19.1
N2	45	50.56
N3	3	3.37
NX	2	2.25
Metastasis (M)		
M0	75	84.27
Mx	14	15.73
Treatment details		
Dose	67.68 ± 2.79	
Fraction	33.84 ± 1.39	
Cisplatin + Radiation	79 (88.75%)	
Radiation	10 (11.25%)	
LDH		
IU/LBefore the beginning of IR (day 1) [before exposure to the first fraction]	368.13 ± 19.56	
After exposure to 2 Gy (24 hours after the first fraction [before exposure to the second fraction]	387.11 ± 18.98 $P < 0.001$	

LDH: Lactate dehydrogenase; IU/L: International Units/ liter; IR: Irradiation; Gy: Gray

increase (18.92 ± 8.98) in the values on day 2 (387.11 ± 18.98 IU/L) when compared to the base line day 1 (368.13 ± 19.56 IU/L), which was statistically significant (t = 20.06 and $P < 0.001$) [Table 2]. The difference between LDH values and severity of dermatitis [mild (grades 1 and 2) vs. severe (grades 3 and 4)] was significant (P value = 0.019; R = 0.24) (Figure 2).

Discussion

The results of the study clearly indicated that the severity of dermatitis increased with exposure

to radiation (Figure 1) and that the differences concerning values in salivary LDH were correlated with the severity of dermatitis (Figure 2). These findings suggested the importance of salivary LDH in predicting radiation-induced side-effects and that it could contribute to earlier diagnosis.[23,24-26] In clinics, in the treatment of HNC, when planned with curative intent, a radiation dose of 60-74 Gy is delivered in 2 Gy fractions 5 days/week for 6 to 7 consecutive weeks and usually severe dermatitis is seen in week four to seven.[26] With regard to the report on the correlation between the difference in salivary LDH (Day 2 - day 1) and the severity of dermatitis [mild (grade 1 and 2) vs. severe (grade 3 and 4)] significant results ($P = 0.019$; $R = 0.24$; Figure 2) were obtained. To the best of the authors' knowledge, there are no reports on the association between salivary LDH and radiation-induced dermatitis. However, reports have suggested that association between atopic dermatitis and serum LDH; the authors also observed that the SCORAD indices were positively correlated with the serum LDH levels ($r = 0.46$, $P < 0.05$).[27] Additionally, animal studies have shown that serum LDH B4 isozyme levels were useful in predicting early diagnosis of radiodermatitis in hairless mice (SKH1-hr).[28]

LDH is present in the cytoplasm of the cell and is a universal and sensitive marker of cell turnover.[29,30] Meanwhile, exposure of the cell to a cytotoxic agent leads to cell rupture and release of LDH into the extracellular environment.[30] Metabolism of cancer cells differs from that of normal cells. Cancer cells preferentially metabolize glucose by glycolysis to generate energy even in the presence of adequate oxygen, and LDH catalyzes the additional step of glycolysis that is predominant under anaerobic conditions.[31] To substantiate the role of LDH in radiation, studies with mammalian cells in vitro have shown that in exposure to external beam radiation, LDH increases in the cultured media;[8,9] this has been observed in serum of the exposed laboratory animals,[10-12] and in humans undergoing curative radiotherapy for their cancer.[13, 14] Increased serum LDH has been proposed as a prognostic marker in colorectal cancer,[32] nasopharyngeal carcinoma,[33] lung cancer,[34] breast cancer,[35] prostate cancer,[36] germ cell cancer,[37] and melanoma.[38] Previous studies with serum LDH have reported it to be useful as a prognostic marker ranging from 220 IU/L in metastatic renal cell carcinoma to 470 IU/L in metastatic pancreatic cancer, indicating its usefulness.[39,40]

Over the recent past years, saliva as a diagnostic tool, has attracted a great deal of attention. This is principally since the use of saliva as a diagnostic fluid has distinct advantages, the most important one being the non-invasiveness of its collection, no need for skilled personnel for collection, and suitability for repeated collections with the least compliance problems.[15, 18] Chemically, saliva is rich in glycoproteins, enzymes, oxidants, antioxidants, and many other molecules whose analysis indicates not only oral health, but also systemic condition/illness of the individual.[41] Salivary LDH has been analyzed in oral cancer[42,43] and studies have shown increased salivary LDH. A meta-analysis of 13 case control studies on oral cancer revealed significantly increased salivary LDH in oral cancer when compared to oral leukoplakia and healthy controls.[43] However, with regard to the correlation between salivary LDH and radiation-induced dermatitis in HNCs, this is the first article addressing the aspect. According to the results obtained herein, we could suggest that salivary LDH holds promising results as a predictive biomarker of radiation-induced damage to the skin in patients undergoing curative radiotherapy for HNC.

Nonetheless, there is a limitation in this research; we considered only one time point post-irradiation (24 h after exposure to 2 Gy of radiation). Studies should be planned to understand the most effective saliva sampling time point during the course of the curative treatment. Moreover, which isoform of salivary LDH is of value as a prognostic marker should also be investigated. The outcome of the planned extended study would contribute to understanding the optimal harvesting time for the assay to be performed for developing salivary LDH as a predictive assay.

Conclusion

The study was based on the hypothesis that during the course of the curative radiotherapy, both skin and buccal cells are affected. Also, radiosensitivity is intrinsic factor and is similar across the tissues in the field of radiation in an individual when compared to another. The observation that salivary LDH correlated with dermatitis validates the hypothesis and is a valuable non-invasive assay end point in clinics.

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Conflict of Interest

None declared.

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