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Cytochrome C as a marker of Apoptotic Cell Death after Chemo-Radiotherapy in Locally Advanced Head and Neck Cancer Patients

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Abstract

Background: Cytochrome C is a key signalling molecule in apoptosis, and its release from mitochondria into the cytosol is an important step, so serum Cytochrome C levels can be used as a marker to assess treatment response in cancer patients, but there is a lack of research data on the effect of chemo-radiotherapy on serum Cytochrome C levels and their correlation with treatment response. Therefore, this study was conducted to determine serum Cytochrome C levels before and after chemo-radiotherapy in patients with locally advanced head and neck cancers.

Aims and Objectives: To determine the role of serum Cytochrome C as a prognostic marker of apoptotic cell death after chemo-radiotherapy in locally advanced head and neck cancer patients. To measure serum Cytochrome C and serum LDH levels before and after one and three months of completion of chemo-radiotherapy (CRT) in patients with locally advanced head and neck cancer (LAHNC).

Materials and Methods: After getting permission from the Ethics Committee, patients with locally advanced biopsy confirmed head and neck cancer were included in this study. All routine biochemical and haematological parameters, Cytochrome C and serum LDH were analysed before starting and at one and three months after the completion of chemo-radiotherapy. A repeated measures ANOVA test was applied to find out the significance of the difference in serum Cytochrome C and serum LDH levels before and after one and three months of chemo-radiotherapy in each group to see the trend of their levels of Group I (complete response),

Group II (partial response), Group III (progressive disease), and Group IV (stable disease).

Results: Analysis of a total of 25 patients with locally advanced head and neck cancer was done based on the RECIST (Response Evaluation Criteria in Solid Tumours) criteria after 3 months of treatment completion. Patients have shown statistically significant decreases in serum LDH **(p value <0.05)** at one and three months in patients with complete responses after three months of chemo-radiotherapy.There was highly significant (p **value <0.05) increase** in levels of serum Cytochrome C at one month and three months in groups I and II. But there was no statistically significant difference **(p value > 0.05)** in levels of serum Cytochrome C at one month and three months in groups III and IV, so no change was observed in serum Cytochrome C levels before and after chemo-radiotherapy in stable disease patients.

Conclusions: Overall results showed that Cytochrome C levels increased significantly after treatment in patients with a good response, while LDH levels decreased significantly in patients with a complete response in head and neck cancer patients. So this study suggests that Cytochrome C can be a useful prognostic marker for detecting apoptosis and assessing treatment efficacy in locally advanced head and neck cancer. However, further research with larger sample sizes and other apoptosis mediators is needed to draw definitive conclusions.

Keywords: Apoptosis, Cytochrome C, Head and Neck Cancer, LDH, Prognostic Marker, Treatment Response.

Introduction

Head and neck cancers (HNCs) are a diverse group of malignant tumours that arise from the upper aerodigestive tract. Most of these cancers are squamous cell carcinomas and have different epidemiology, etiology, and treatment options. Treatment plans depend on factors such as tumour location, stage, and the patient's age and general health[1,2].

Chemoradiotherapy is a commonly used treatment for HNCs and works by triggering apoptosis, a programmed cell death mechanism. Cytochrome C is a key signalling molecule in apoptosis, and its release from mitochondria into the cytosol is an important step in the process[1,2,3.]

Studies have shown that serum cytochrome C levels can be used as a marker to assess treatment response in cancer patients, but there is a lack of research on the effect of chemo-radiotherapy on serum cytochrome C levels and their correlation with treatment response. Therefore, this study was conducted to determine serum cytochrome C levels before and after chemo-radiotherapy in patients with locally advanced head and neck cancers and to investigate their correlation with treatment response[1,2,3].

So this research was carried out with the intention of understanding the relationship between serum cytochrome C and apoptosis, which can aid in designing targeted therapies for cancer patients undergoing chemo-radiotherapy.

Methods

After permission from the Institutional Ethics Committee for Biomedical and Health Research, this hospital-based prospective analytical study was carried out at the Clinical Chemistry Laboratory in close collaboration with the Department of Radiation Oncology, Sir Sayajirao General (S.S.G.) Hospital, and Medical College Baroda, Vadodara.

This study was done over a period of six months, from July 2021 to December 2021. A total of 25 patients with

locally advanced head and neck cancer were included in this study.

Aim

To determine the role of Serum Cytochrome C as a marker of apoptotic cell death after chemo-radiotherapy in locally advanced head and neck cancer patients.

Objectives

1. To measure the Serum Cytochrome C & Serum LDH levels before and after one & three month of completion of chemo-radiotherapy (CRT) in patients of locally advanced head and neck cancer (LAHNC).
2. To evaluate the AJCC (American Joint Committee on Cancer, 8th edition) staging of locally advanced head & neck cancer before and after completion of CRT.
3. To compare Serum Cytochrome C and Serum LDH levels between stages of LAHNC before beginning of CRT.
4. To group the patients on the basis of RECIST criteria (Response Evaluation Criteria in Solid Tumours) for response evaluation after 3 month of completion of CRT and correlate it with trend of Serum Cytochrome C and Serum LDH levels.

Inclusion Criteria

1) All clinically and pathologically confirmed cases of locally advanced head & neck cancer (stage III, IVA & IVB), >18 years of age, who are eligible for definitive chemo-radiotherapy.
2) Chemo-radiotherapy naïve patients.

Exclusion Criteria

1) All recurrent cases of locally advanced head & neck cancers.
2) Patients with distant metastasis (stage IVC).
3) All patients who lost to follow up.

Method of Data Collection

Total 25 patients of locally advanced head and neck cancer who presented in S.S.G hospital's ENT department and managed under Radiation Oncology Department between July 2021 to December 2021 were included in this study. These patients were first evaluated by ENT department and after endoscopic guided biopsy and histo-pathological confirmation of cancer they were referred to Radiation Oncology Department. Patients were categorized into clinical staging by history & physical examination, locoregional examination including Hopkin's endoscopy and all routine pathological investigations and radiological examination (CECT Scan head & neck and/or whole-body PET-CT Scan). The final fitness for chemo-radiotherapy was also done by Radiation Oncology Department depending on performance status aspect ECOG (Eastern Cooperative Oncology Group) Criteria.

Informed consent of subjects was obtained for participation in the study and for blood collection. Patient's detailed history was taken and examination findings were noted from patient's clinical records as per proforma. Apart from routine investigations, Serum Cytochrome C and Serum LDH were also tested.

3-4 ml blood samples were collected in plain vacutainer, 1-2 ml blood sample in fluoride vacutainer and 2-4 ml blood sample in EDTA vacutainer for the measurement of biochemical and haematological parameters.

All routine biochemical & haematological parameters and Serum LDH were analyzed at the same time after obtaining the samples of patients before starting their chemo-radiotherapy. Serum was separated within an hour and stored at -20 °C temperature for serum Cytochrome C. On follow up at one and three month of completion of chemo-radiotherapy, in addition to

investigations advised by treating physician, serum Cytochrome C and Serum LDH were also tested.

Various investigations done were as follows;

Hematogram Profile

Biochemical Investigations

1. **Hematogram Profile:**
   a) Haemoglobin (Hb)
   b) Total Leukocyte Count (TC)
   c) Differential Leukocyte Count (DC)
   d) Platelet Count (PC)
2. **Biochemical Investigations:**

Routine tests

a) Plasma Random Blood Glucose
b) Serum Urea
c) Serum Creatinine
d) Serum Bilirubin
e) Serum Alanine Aminotransferase (ALT)
f) Serum Aspartate Aminotransferase (AST)
g) Serum Alkaline Phosphatase (ALP)
h) Serum Total Protein
i) Serum Albumin

Special Tests

a) Serum Lactate Dehydrogenase (LDH)
b) Serum Cytochrome C.

Hb, TC, DC and PC were performed on Heamatron automated cell counter for final fitness for chemo-radiotherapy. All the routine biochemical tests and Serum LDH were performed on ERBA XL-640 Fully Automated Biochemistry Analyzer.

Serum Cytochrome C was done by ELISA on Microlab ELISA washer and Alere ELISA reader. The method of performance of each tests were as follows;

I.	Serum Cytochrome C: Sandwich ELISA
II.	Serum LDH: P to L, UV Kinetic Method

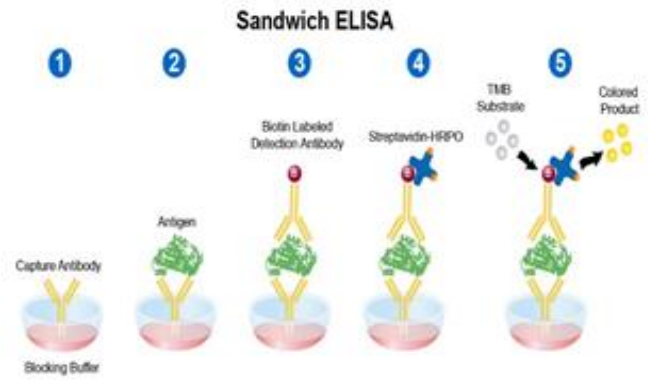


Figure: 1: Steps of Sandwich ELISA for Serum Cytochrome C

Estimation of Serum Cytochrome C was done by Sandwich Enzyme Linked Immunosorbent Assay method[i]. Estimation of Serum Lactate Dehydrogenase (LDH) was done by UV Kinetic Method[2,3].

We have compared initial levels of Serum Cytochrome C and Serum LDH in stage III, IVA and IVB of locally advanced head and neck cancer based on AJCC (American Joint Committee of Cancer) staging.

After three month of completion of chemo-radiotherapy, CECT scan of Head and neck was done and evaluated by Radiation Oncology Department. According to RECIST (Response Evaluation Criteria in Solid Tumours) criteria, the patients were categorized into 4 groups as under.

Group I: Complete Response

- Disappearance of all target lesions.

Group II: Partial Response

- ≥30% decrease in the sum of the longest diameters of target lesions compared with baseline.

Group III: Progressive Disease

- ≥20% increase in the sum of the longest diameter of target lesions compared with the smallest-sum longest diameter recorded or the appearance of one or more new lesions.

Group IV: Stable Disease

- Neither partial response (PR) nor progressive disease (PD).
- We compared serum Cytochrome C & Serum LDH level before and after one & three month of treatment in each group.

Ethics

Study was conducted after approval and ethical clearance obtained from Institutional Ethics Committee for Biomedical and Health Research (IECBHR) of Medical College and S.S.G. Hospital Baroda.

Protection of Patients' Rights of Privacy: Authors have followed the Indian Council of Medical Research (ICMR) issued guidelines and International Council for Harmonization (ICH) Guideline for Good Clinical Practice (GCP) to protect the privacy rights of patients in cancer research. Here are some of the measures that can be taken to ensure the protection of patients' rights of privacy.

Ethics committee approval: This research studies involving human subjects, including cancer research, was approved by an ethics committee - IECBHR. The ethics committee had reviewed the study protocol to ensure that the privacy and rights of the patients are protected.(Approval No. IECBHR / 115 - 2021 / Date: 03/09/2021).

Informed consent: Before enrolling patients in any research study, informed consent was obtained from them. The consent form consisted information about the nature and purpose of the research, the procedures involved, the risks and benefits, and the measures taken to protect confidentiality.

Confidentiality: All personal identifying information about the patients, including their name, address, and medical records, was kept confidential.

Access to data: Only authorized personnel have access to the data collected during the research study. Researchers have ensured that the data is stored securely and only accessed by individuals who need it for the study.

Statistics

Shapiro Wilk test was applied to see the normal distribution of the data and statistically described as Mean ± SD.

One-way ANOVA test was applied to find out the significance of difference of initial levels of serum LDH and serum Cytochrome C in locally advanced head and neck cancer stages (stage III, stage IVA & stage IVB).

Repeated measures ANOVA test was applied to find out significance of difference in serum Cytochrome C and serum LDH levels before and after one & three month of chemo-radiotherapy in each group and see the trend of their levels. (Group I, II, III & IV).

Interpretation was done according to p-values as follows:

– $P < 0.05$ was considered significant
– $P < 0.001$ was considered highly significant
– $P \geq 0.05$ was considered not significant

All statistical analysis was done using free version of online MedCalc software.

Results

This prospective analytical study was done over a period of 6 months, from July 2021 to December 2021 in Clinical Chemistry Laboratory in collaboration with Radiation Oncology Department, S.S.G. Hospital, Vadodara. Total 26 cases of locally advanced head and neck cancer, more than 18 years old were enrolled in our

study. Out of 26 patients, one patient was lost to follow up and so he was excluded from the study. For all the 25 patients included in our study, detailed history was taken, results of physical examination and radiological & routine laboratory investigations were noted from patient's clinical record as per proforma. Serum Cytochrome C and Serum LDH were estimated in Clinical Chemistry Laboratory before start of chemo-radiotherapy and after one and three month of completion of chemo-radiotherapy. On, Shapiro Wilk test, all data were found to be normally distributed. So, data has been statistically described in terms of mean ± SD.

The observations made with respect to various aspects of the study are as follows.

1. Age &Gender wise distribution

Table: 1 Age and gender distribution Amongst 25 Patients of Head and Neck Cancer

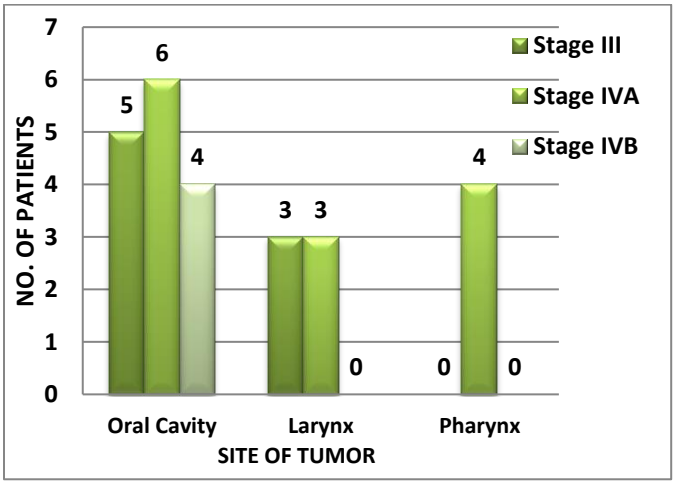
Gender Distribution	MALE	FEMALE	Total
	20	05	25
Age (in years) Mean ± SD	56 ± 10.41	42 ± 15.3	53.1 ± 12.37

Table 1 shows that out of 25 patients of locally advanced head and neck cancer, 20 were males and 5 were females. Mean age of all patients was 53.1±12.37 (40-65) year, for male mean age was a 56±10.41 (46-66) year and for female mean age was 42± 15.3 (27-57) years.

2. Site wise & stage wise distribution of Locally Advanced Head & Neck Cancer (LAHNC) Patients.

Table 2. Site wise & stage wise distribution of Locally Advanced Head & Neck Cancer (LAHNC) Patients.

Sr. No.	Site of Tumor	Stages of LAHNC			Total no. of Patients (Site wise)
		III	IVA	IVB	
1	Oral Cavity	05	06	04	15(60%)
2	Larynx	03	03	00	06(24%)
3	Pharynx	00	04	00	04(16%)
Total no. of Patients (Stage wise)		08	13	04	25(100%)



Graph: 1 Distributions of Patients According to Site of Tumor and Stages of LAHNC

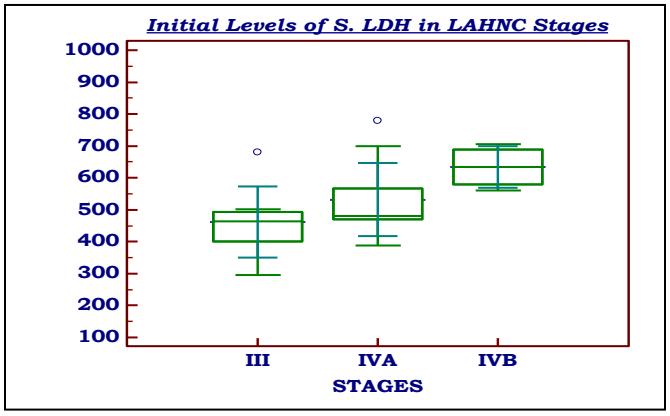
According to table 2 & graph 1, oral cavity cancer was most common site of LAHNC and stage IVA was most common stage among 25 patients.

3. Comparison of Initial Level of Serum LDH and Serum Cytochrome C

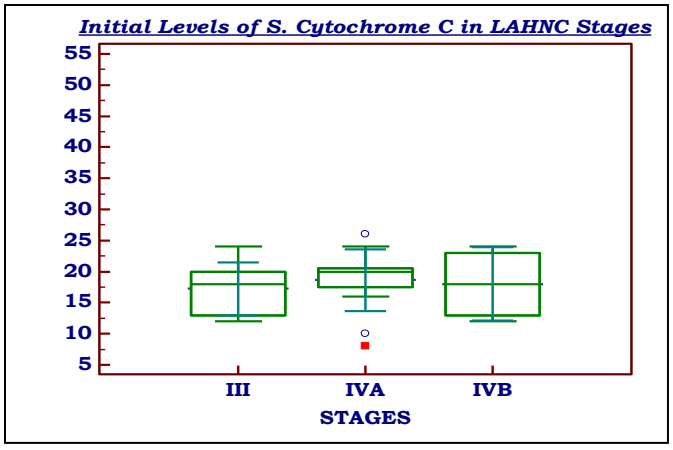
Table: 3 and Graph: 2 & 3 show comparison of initial level of Serum LDH and serum Cytochrome C among various stages of LAHNCs.

Table: 3 Stage wise Initial Levels of Serum LDH and Serum Cytochrome C in cases of LAHNC.				
Parameters (Mean ± SD)	LAHNCs Stages			p Value
	Stage III	Stage IVA	Stage IVB	
Serum LDH in IU/L	461.3 ± 111.1	531.4 ± 114.3	634.0 ± 66.1	0.049
Serum Cytochrome C in ng/ml	17.2 ± 4.26	18.6 ± 4.99	18 ± 5.88	0.827

Table 3 & Graph 2 show that mean ± SD of initial levels of serum LDH in stage III was 461.3 ± 111.1 U/L, in stage IVA was 531.4 ± 114.3 U/L and in Stage IVB was 634.0 ± 66.1 U/L. There was statistical significant difference of initial level of serum LDH in LAHNC stages and levels were higher in more advanced stages.



Graph: 2 Stage wise Initial Levels of Serum LDH in Cases of LAHNC



Graph: 3 Stage wise Initial Levels of Serum Cytochrome C in Cases of LAHNC

Table 3 & Graph 3 show that mean ± SD of initial levels of serum Cytochrome C in stage III was 17.2 ± 4.26 ng/ml, in stage IVA was 18.6 ± 4.99 ng/ml and in Stage IVB was 18 ± 5.88 ng/ml. There was no statistical significant difference of initial level of serum Cytochrome C in various stages of LAHNC.

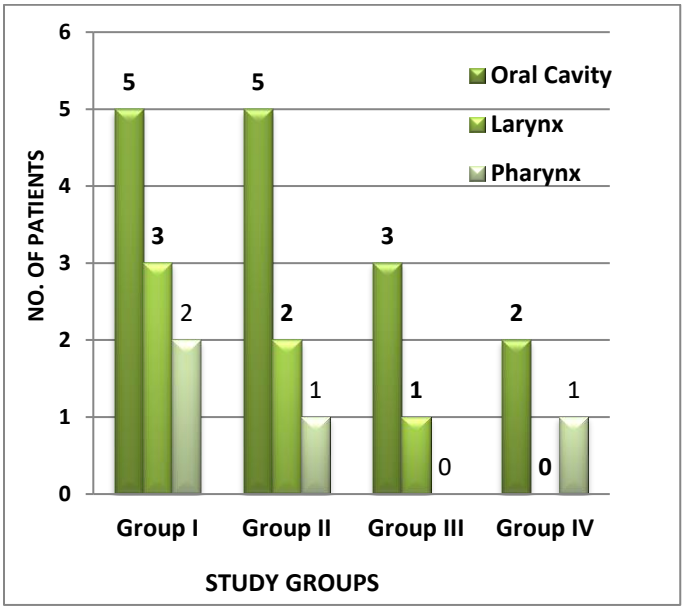
After 3 month of completion of treatment, 25 patients were divided into four groups, based on the RECIST (Response Evaluation Criteria in Solid Tumours) criteria, as follows;

- **Group I:** Complete Response
- **Group II:** Partial Response
- **Group III:** Progressive Disease
- **Group IV:** Stable Disease

4. Distribution of Patients According to Site of Tumor

Table: 4 & graph: 4 show distribution of patients according to site tumor in four groups. They show that most common site for LAHNC in study group was oral cavity (Group I=5, group II=5, group III=3 & group IV=2) followed by larynx (Group I=3, group II=2 & group III=1 & group IV=0) and pharynx (Group I=2, group II=1, group III=0 & group IV=1).

Table: 4 Distribution of Patients According to Site of Tumor in Group I, II, III & IV				
STUDY GROUPS	No. of patients			Total no. of Patients in each Group
	Oral Cavity	Larynx	Pharynx	
Group I	05	03	02	10
Group II	05	02	01	08
Group III	03	01	00	04
Group IV	02	00	01	03
Total No. of Patients	15	06	04	25



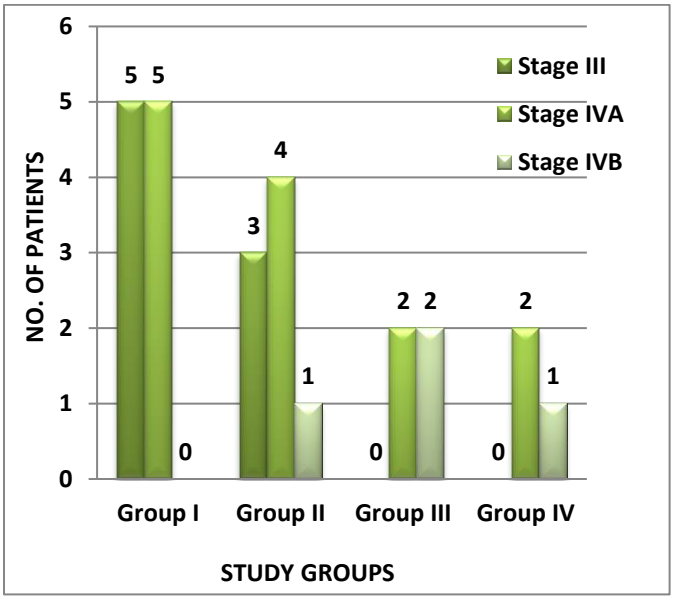
Graph: 4 Distributions of Patients According to Site of Tumor in Group I, II, III & IV

5. Distribution of Patients According to Initial Staging

Table: 5 & Graph: 5 show distribution of patients according to initial staging in four groups.

Table: 5 Distributions of Patients According to Initial Staging in Group I, II, III & IV

STUDY GROUPS	NO. OF PATIENTS			Total No. of Patients
	Stage III	Stage IVA	Stage IVB	
Group I	05	05	00	10
Group II	03	04	01	08
Group III	00	02	02	04
Group IV	00	02	01	03
Total No. of Patients	08	13	04	25



Graph: 5 Distribution of Patients According to Initial Staging in Group I, II, III & IV

Table 5 & graph 5 show that group I (n=10) includes stage III (n=5) & stage IVA (n=5) patients, group II (n=8) includes stage III (n=3), stage IVA (n=4) & stage IVB (n=1), group III (n=4) includes stage IVA (n=2) & stage IVB (n=2) and group IV (n=3) includes stage IVA (n=2) & stage IVB (n=1).

6. Comparison of Serum LDH Levels

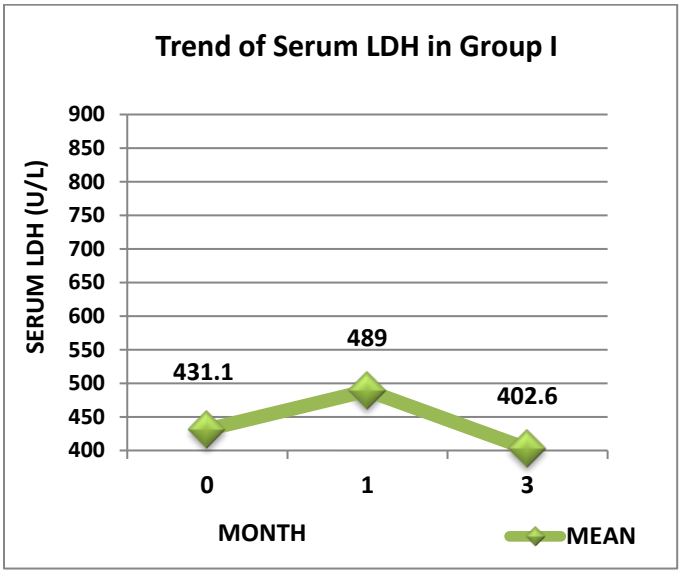
Table: 6& graph: 6, 7, 8 & 9 show levels of Serum LDH in four groups of LAHNC before chemo-radiotherapy and after one and three month of completion of chemo-radiotherapy.

Table: 6 Trends of Serum LDH Levels in Group I, II, III, and IV

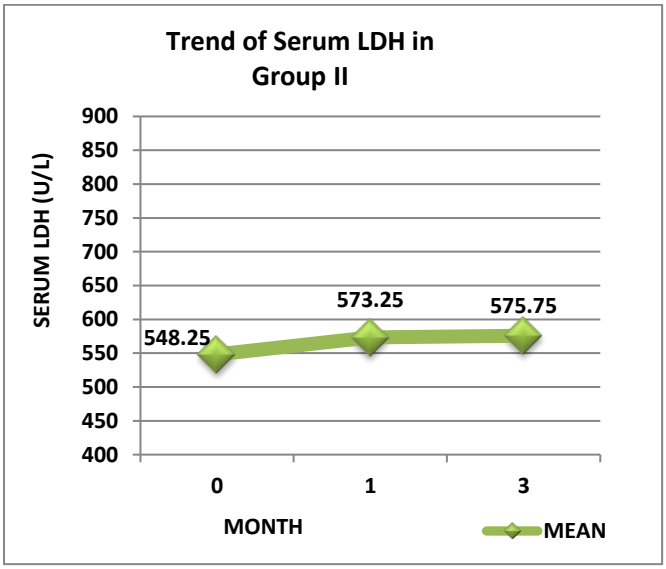
STUDY GROUPS	No. of Patients	Initial Serum LDH	1 Month Serum LDH	3 Month Serum LDH	p-Value
Group I (Complete Response)	08	431.1 ± 61.7	489± 39.2	402.6 ± 80.8	0.001
Group II (Partial Response)	10	548.2 ±	573.2 ± 88.0	575.7 ± 64.4	0.588

		86.3			
Group III (Progressive Disease)	04	680.7 ± 85.5	775.2 ± 27.7	781.2 ± 15.6	0.055
Group IV (Stable Disease)	03	629.6 ± 37.8	660± 27.8	634± 48.0	0.470

Table 6 & graph 6 show statistical significant difference (p value=0.001) in Serum LDH initial, 1 month & 3-month level in group I having Mean ± SD, 431.1 ± 61.7, 489 ± 39.2 & 402.6 ± 80.8. There is a decrease in Serum LDH levels in patients with complete response after 3 month of completion of chemo-radiotherapy.



Graph: 6 Trend of Serum LDH levels in Group I (Complete Response)



Graph: 7 Trend of Serum LDH levels in Group II (Partial Response)

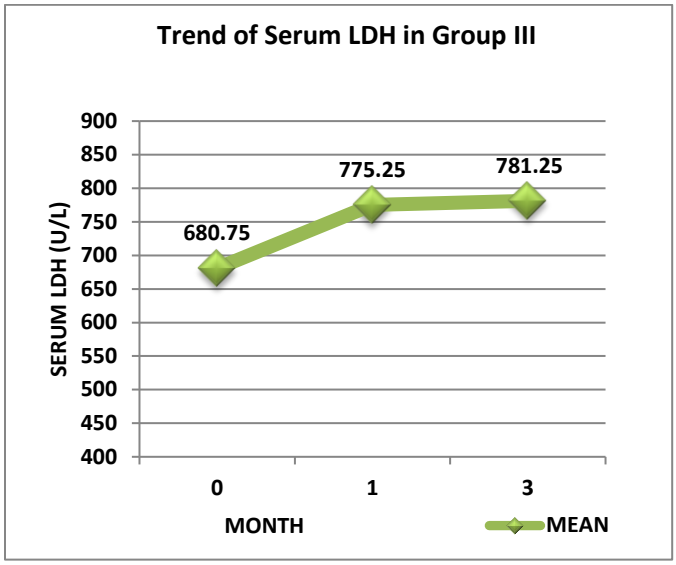
Table 6 & graph 7 show no statistical significant difference **(p value=0.588)** in Serum LDH initial, 1 month &3-month level in group II having Mean ± SD, 548.2 ± 86.3, 573.2 ± 88.0 & 575.7 ± 64.4.

Table 6 & graph 8 show no statistical significant difference (p value=0.05) in Serum LDH initial, 1 month & 3-month level in group III having Mean ± SD, 680.7 ± 85.5, 775.2 ± 27.7 & 781.2 ± 15.6 though Serum LDH levels increase with progression of the disease.

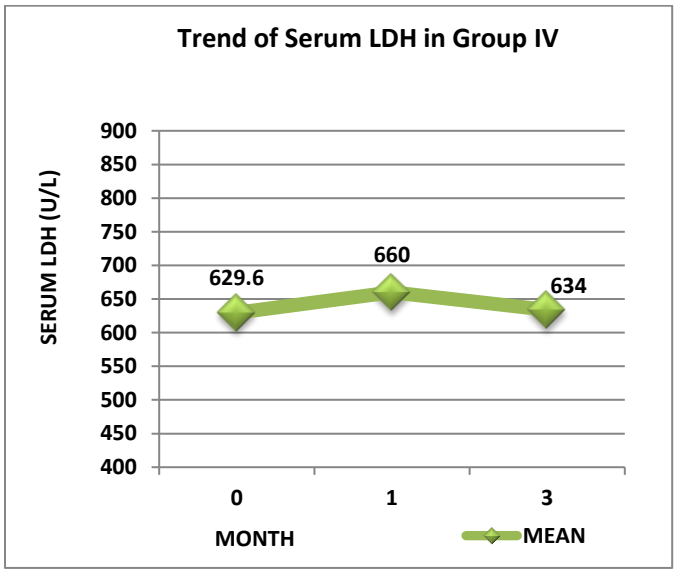
Table 6 & graph 9 show no statistical significant difference (p value=0.470) in Serum LDH initial, 1 month & 3-month level in group IV having Mean ± SD, 629.6 ± 37.8, 660 ± 27.8, 634 ± 48.0.

7. Comparison of Serum Cytochrome C Levels

Table: 7 & graph: 10, 11, 12 & 13 show levels of Serum Cytochrome C in four groups of LAHNC before chemo-radiotherapy and after one and three month of completion of chemo-radiotherapy.



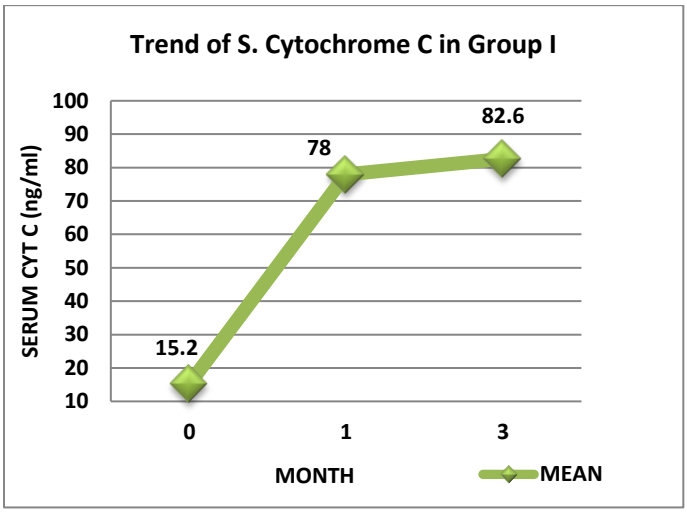
Graph: 8 Trend of Serum LDH levels in Group III (Progressive Disease)



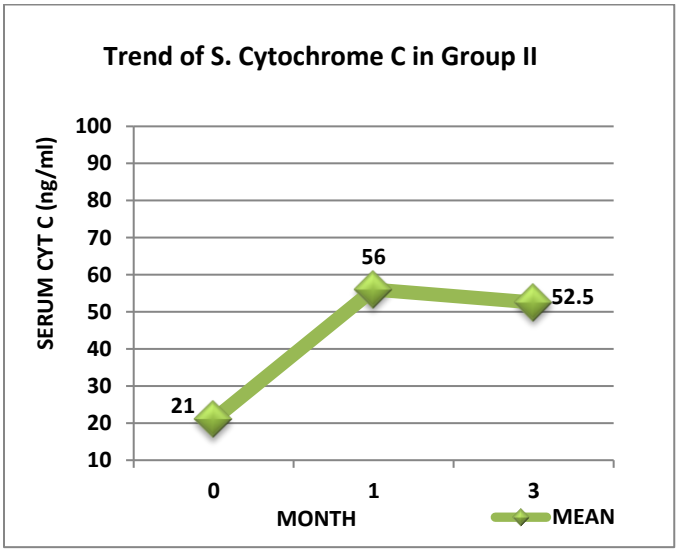
Graph: 9 Trend of Serum LDH levels in Group IV (Stable Disease)

Table: 7 Trends of Serum Cytochrome C Levelsin Group I, II, III, and IV

STUDY GROUPS	No. of Patients	Initial Serum Cytochrome C	1 Month Serum Cytochrome C	3 Month Serum Cytochrome C	p-Value
Group I (Complete Response)	08	15.2 ± 4.73	78 ± 11.96	82.6 ± 12.40	<0.001
Group II (Partial Response)	10	21 ± 2.61	56 ± 8.61	52.5 ± 11.94	0.002
Group III (Progressive Disease)	04	18 ± 6.32	23 ± 3.46	19 ± 8.71	0.135
Group IV (Stable Disease)	03	20 ± 2.00	23.3 ± 4.1	24.6 ± 4.16	0.729



Graph: 10 Trend of Serum Cytochrome C levels in Group I (Complete Response)



Graph: 11 Trend of Serum Cytochrome C levels in Group II (Partial Response)

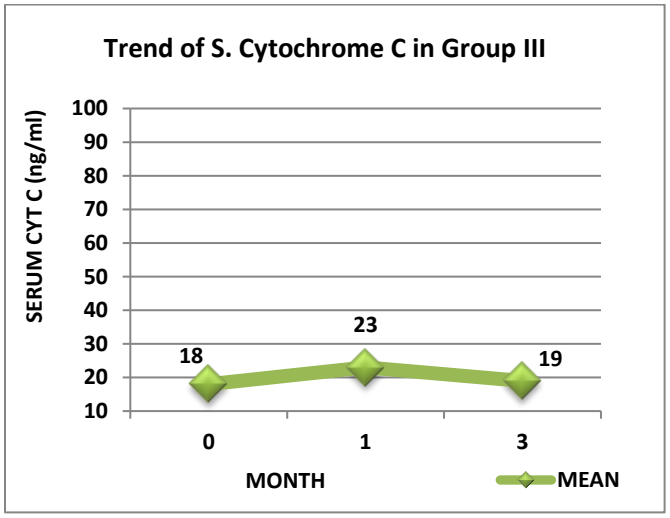
Table 7 & graph 10 show highly statistical significant difference (p value=<0.001) in levels of Serum Cytochrome C initial, 1 month & 3 month in group I

having mean ± SD, 15.2 ± 4.73, 78 ± 11.93 & 82.6 ± 12.40.

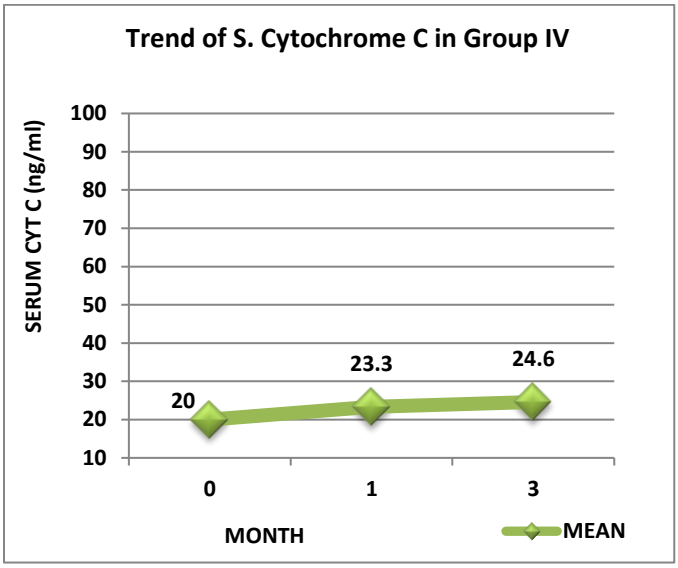
Table 7 & graph 11show statistical significant difference **(p value=0.002)** in levels of Serum Cytochrome C initial, 1 month & 3 month in group II having mean ± SD, 21 ± 2.61, 56 ± 8.61 & 52.5 ± 11.94.

Table 7 & graph 12 show no statistical significant difference (p value=0.135) in levels of Serum Cytochrome C initial, 1 month & 3 month in group III having mean ± SD, 18 ± 6.32, 23 ± 3.46 & 19 ± 8.71. No change in serum Cytochrome levels before and after chemo-radiotherapy in progressive disease patients.

Table 7 & graph 13 show no statistical significant difference (p value=0.729) in levels of Serum Cytochrome initial, 1 month & 3 month in group IV having mean ± SD, 20 ± 2.00, 23.3 ± 4.1 & 24.6 ± 4.16. No change in serum Cytochrome levels before and after chemo-radiotherapy in stable disease patients.



Graph: 12 Trend of Serum Cytochrome C levels in Group III (Progressive Disease)



Graph: 13 Trend of Serum Cytochrome C levels in Group III (Progressive Disease)

In our study, we observed that serum Cytochrome C levels before and after one and three month of completion of chemo-radiotherapy trend was significant in group I & II while Serum Cytochrome C levels were not changed in group III & IV. Serum LDH levels before and after one and three month of completion of chemo-radiotherapy trend was significant in group I while not changed in group II, III and IV.

Discussion

The annual incidence of head and neck cancers worldwide is more than 5,50,000 cases with around 3,00,000 deaths each year[2]. It is the sixth leading cancer worldwide[ii]. In India, it accounts for one fourth of all male cancers and one tenth of all female cancers[3]. The treatment options for locally advanced head and neck cancer traditionally include surgery followed by adjuvant radiotherapy (S+RT) or adjuvant chemo-radiotherapy (S+CRT), or only definitive CRT[4]. Despite improving treatment outcomes with this multimodality treatment, there is a high rate of death.

Monitoring during the treatment and follow up by biochemical markers can be helpful to find out the disease progression and response to the treatment and can improve the overall survival rate in male as well as female.

Cytochrome C, the key regulator of apoptotic pathway, is released not only into cytoplasm, but also leaves the cell. This externalization of Cytochrome C is an early and apoptosis-specific event, which occurs during chemo-radiotherapy of cancer patients. The assessment of Cytochrome C release may therefore provide useful assay to detect apoptosis and to determine cell turnover and treatment efficacy in chemo-radiotherapy.

This is a hospital based prospective analytical study carried out at Clinical Chemistry Laboratory in collaboration with Radiation Oncology Department, S.S.G. Hospital & Medical College Baroda, Vadodara. The aim of this study was to determine the role of Cytochrome C as a marker of apoptotic cell death after chemo-radiotherapy in locally advanced head & neck cancer patients.

The study included 25 patients of locally advanced head and neck cancer. In our study group mean age for male (n=20) was 56±10.41 (46-65) years and for Female (n=5) was 42± 15.3 (27-57) years. Head and neck cancers were traditionally being thought to be as a disease mainly affecting people of old age group (>66 years)[iii,iv]. In the present study, the trend has shifted to lower age group. This usually attributed to indiscriminate usage of substances, mainly tobacco, tobacco related products and alcohol, over a prolonged period of time, which leads to genetic damage.

In this study, in the term of gender distribution, out of 25 patients 20 were males and 5 were females with ratio of 4:1. This is in agreement with sex ratio given in various studies. JivranjaniParimal J et alfound male: female ratio to be 4.5:1. Similarly, Larizadeh et al observed same male: female ratio (4.5:1)[5,6].This suggested that head and neck cancer is more predominant in males than in females.

Further, in our study the commonest site was observed to be oral cavity in both males (n=20) and females (n=5). Oral cavity accounted for most prevailing cancer of total head and neck cancer cases (n=15/60%) followed by larynx (n=6/24%) and pharynx (n=4/16%). This is in agreement to study done by P.K. Sahu et al. and Japhet M et al[5]. They both found the oral cavity cancer is the most prevalent form of head and neck cancer[v]. But at variance, Bhattacharjee et al[7] reported the oral cavity to be the third common head and neck cancer and pharyngeal cancer to be most common cancer and Ologe FE et al [6] found the nose and paranasal sinuses cancers to be the highest number of cases followed by nasopharyngeal cancer in Nigeria Haraf DJ et al[16] reported increase incidence of HPV related cancer mainly attributed to the unusual sexual practice in the western world. In India HPV prevalence ranges from 33.3 % in East to 67% in south and 15% in west[16].

In this study, we included locally advanced head and neck cancer (LAHNC) patients (Stage III, IVA and IVB). Out of 25 patients, 8 patients were in stage III, 13 were in stage IVA & 4 were in stage IVB. Highest number were found in most advanced stage (IV) among the cases included in our study at the time of diagnosis which may be attributed to be the fact that the majority of patients in this study presented late to our health facility. The majority of patients in our environment, especially those in the rural areas lack awareness about modern health facilities due to high poverty level and this is further compounded by harmful traditional beliefs

and practices which make them visit the herbalists for solutions to their health problems so that by the time they present to us, their tumors would have reached advanced stages and hence a poor outcome in management.

The Mean ± SD of initial levels of serum LDH in stage III was 461 ± 111.1 U/L, in stage IVA was 531.4 ± 114.3 U/L and in stage IVB was 531.4 ± 114.3 U/L. There was statistical significant difference in initial levels of serum LDH in LAHNC stages. Our findings are in agreement with literature which states that serum LDH represents tumor load or burden. WahyuWulaningsih et al.[8] (2015) observed an inverse association between baseline serum LDH and survival following cancer diagnosis. Liu R et al (2016) reported that serum LDH could be a marker of tumor burden for advanced cancer patients[17].

The mean ± SD of initial level of serum Cytochrome C in stage III was 17.2 ± 4.26 ng/ml, in stage IVA was 18.6 ± 4.99 ng/ml and in stage IVB was 18 ± 5.88 ng/ml showing no statistical significant difference in initial serum Cytochrome C level and LAHNC stages. In literature search we couldn't find any documented reference range for serum Cytochrome C in healthy subjects. Mei Afify et al reported mean ± SD for serum Cytochrome C in hepatocellular carcinoma was 11.94 ± 4.69 ng/ml, in chronic hepatitis was 7.34 ± 1.89 ng/ml and in healthy control was 4.09 ± 0.92 ng/ml[17].

We divided 25 patients of locally advanced head and neck cancer into 4 groups based on RECIST criteria depending on CECT scan which was done by treating clinician. Among 25 patients 10 were in group I (Complete response), 8 were in group II (Partial response), 4 were in group III (Progressive disease) and 3 were in group IV (Stable disease).

There was statistical significant difference of serum LDH initial, 1 month and 3 month levels in group I (Complete Response) (p value=0.001) having mean ± SD, 431.1 ± 61.7 U/L, 489 ± 39.2 U/L & 402.6 ± 80.8 U/L respectively. But there was not statistical significant difference in group II (Partial Response) (p value=0.588) having mean ± SD, 548.2 ± 86.3 U/L, 573.2 ± 88.0 U/L & 575.7 ± 64.4 U/L , in group III (Progressive Disease)(p value=0.055) having mean ± SD, 680.7 ± 85.5 U/L, 775.2 ± 27.7 U/L & 781.2 ± 15.6 U/L and in group IV (Stable disease)(p value=0.470) having mean ± SD, 629.6 ± 37.8 U/L, 660 ± 27.8 U/L & 634 ± 48.0 U/L. These results suggest that serum LDH levels indicates tumor load, levels fall with good response to treatment but remaining high in cases with poor response in agreement with Agata Forkasiewicz et al study. They observed that serum LDH, beyond its diagnostic and prognostic role can also be a useful indicator of the effectiveness and efficiency of anticancer therapy and an elevated serum LDH level was found to be an unfavorable indicator for survival in cancer patients, it suggests that serum LDH can be used as a marker of tumor aggressiveness[17].

In this study we found statistically highly significant increase in Serum Cytochrome C levels after one & three month of treatment in group I (Complete response) (Serum Cytochrome C levels before treatment, after 1 month & 3 month of treatment being 15.2 ± 4.73 ng/ml, 78 ± 11.96 ng/ml & 82.6 ± 12.40 ng/ml respectively [p value=<0.001]). In patients with partial response (Group II) also statistical significant increase in Serum Cytochrome C levels. (Serum Cytochrome C levels before treatment, after 1 month & 3 month of treatment being 21 ± 2.61 ng/ml, 56 ± 8.61 ng/ml & 52.5 ± 11.94 ng/ml respectively [p value=0.002]). However, in

patients without good response (Progressive disease [group III] & stable disease [Group IV]) there was no statistically significant increase in Serum Cytochrome C levels. (Serum Cytochrome C levels before treatment, after 1 month & 3 month of treatment in group III was 18 ± 6.32 ng/ml, 23 ± 3.46 ng/ml & 19 ± 8.71 ng/ml respectively [p value=0.135] & in group IV was 20 ± 2.00 ng/ml, 23.3 ± 4.10 ng/ml & 24.6 ± 4.16 ng/ml respectively [p value=0.729]). These data suggest that increased level of Cytochrome C can be a useful marker for response to treatment during the treatment. Andrea Renz et al found high Cytochrome C level during the chemotherapy and they found that majority of patients responded to chemotherapy with increased level of Cytochrome C[17].

Some studies hypothesized that one of the mechanisms of action of CRT is by release of Serum Cytochrome C can serve as activator for caspases and thereby trigger the apoptosis[8]. Our study supports these hypotheses that chemo-radiotherapy causes mitochondria wall damage and release of Cytochrome C that causes apoptosis of malignant cell in cancer patients and provides a useful marker to detect apoptosis and to determine cell turnover and treatment response in many cancers[18,19].

Further Recommendation

So far Cytochrome C is being studied only for research purpose. More studies to understand role of Cytochrome C & other apoptosis mediators in patients on cancer treatment by CT & RT are required so that they can be used as a marker to evaluate response to treatment. Further, better understanding of these mediators will help to devise treatment modalities targeted at these mediators.

Conclusions

This study has identified a co-relation between serum Cytochrome C levels with clinical response in patients with locally advanced head and neck cancer. Overall results showed that Cytochrome C levels increased significantly after treatment in patients with good response, while LDH levels decreased significantly in patients with complete response. This study also suggests that Cytochrome C can be a useful prognostic marker for detecting apoptosis and assessing treatment outcome in locally advanced head and neck cancer patients. However, further research with larger sample sizes and other apoptosis mediators is needed to draw definitive conclusion.

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