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ALTERATION OF SERUM TRANSAMINASES AND GAMMA GLUTAMYL TRANSFERASE IN HEAD AND NECK CANCER

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ABSTRACT

Although there are many studies on circulating tumor markers for breast cancer, germ cell tumors and adenocarcinomas of the colon, prostate and ovary, very few studies on the role of liver enzyme in head neck squamous cell carcinoma (HNSCC) and precancerous conditions are available. As various substances alter quantitatively in the serum during tumor development, we intended to explore the changes in serum aspartate transaminase (AST), serum alanine transaminase (ALT), serum gamma-glutamyl transpeptidase (GGT) and blood protein profile in these groups of patients. We examined these enzymes in histologically confirmed cases of SCC (n=31) and Premalignant lesions (n=30) of head and neck and found that AST, ALT. ANOVA results showed significant elevation of these enzymes in SCC and precancer conditions((p<.0001) . Moreover, mean serum GGT levels were also elevated in HNCA in comparison to control levels. We propose that determination of changes in serum AST, ALT and GGT in HNSCC and premalignant lesions may serve to predict malignant transformation or prognosis of such lesions which are otherwise clinically not always possible to ascertain.

KEYWORDS: **Head and neck cancers, serum aspartate transaminase, serum alanine transaminase, serum gamma-glutamyl transpeptidase, premalignant lesion**

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1. INTRODUCTION

Head and neck cancers (HNCA) are the sixth commonest cancer in the world[1] (Macha et al., 2014). In North-east India, which comprises of the states of Manipur, Mizoram,Tripura, Assam , Nagaland and Arunachal Pradesh, the incidence is reported to be highest in the country (54.48%). [2] Among various factors known to contribute to HNCA, smoking and alcohol consumption in Western countries and use of smokeless tobacco, areca nut and/or betel quid in Southeast Asia are the commonest causes . In this scenario, early HNCA detection and assessment with the help of biomarkers assumes great importance. Although there are many studies on circulating tumor markers for breast cancer, germ cell tumors and adenocarcinomas of the colon, prostate and ovary, very few studies on the role of liver enzyme in head neck squamous cell carcinoma (HNSCC) and precancerous conditions are available. As various substances alter quantitatively in the serum during tumor development, we intended to explore the changes in serum aspartate transaminase (AST), serum alanine transaminase (ALT), serum gamma-glutamyl transpeptidase (GGT) and blood protein profile in these groups of patients. As no previous study on this aspect has been carried out in patients of this region, this study will throw new light on the status of these biochemical parameters as a candidate for HNCA biomarker.

2. MATERIALS AND METHODS

A total of 61 patients took part in the study which was carried out at Silchar Medical College Hospital under Department of Life Sciences, Assam University. Patients with any systemic disease including hepatic and previous cancer treatment were excluded from the study. Thirty one histopathological diagnosed cases of HSCC comprised the of the first group. The second group comprised of thirty cases of Premalignant lesions (viz- verrucous lesion, leukoplakia, erythroplakia, and oral submucous fibrosis. Age and sex matched disease-free subjects were selected for the Control group. We evaluated the relation between serum AST, ALT, GGT, serum Albumin and serum Bilirubin. On obtaining informed–consent from the patients, venous blood samples (5ml) were collected to estimate the biochemical parameters. Serum albumin measurement was done by photometric colour test using Automated Wet Chemistry Analyser (Beckman Coulter Au 400) and Serum Total Protein using Olympus system Calibrator. For serum BLB, GGT, AST and ALT, estimation was done by BECKMAN COULTER “AU 480” autoanalyzer. We considered high serum enzyme level when its level was detected above the cutoff normal range as set by autoanalyzer machine. The normal cut-off range for serum AST and ASLT was 10-37UL-1 and for ALT was GGT 4-38UL-1. Analysis of variance (ANOVA) and Duncan test were used to compare differences between groups and the significance level of $p<.05$ was considered. Statistical tests were done using the GraphPad Instat software, Version 3.05 (GraphPad Inc., CA, USA).The approval of the appropriate Institutional ethical committee of was obtained before conducting the study.

3. OBSERVATIONS & RESULTS

4.1 Patient characteristics

The mean age in SCC, premalignant and the control group were 61.7 years, 53.1 years and 59.4 years respectively. Male: Female ratios for the three groups were 23.8, 26:4 and 18:12 respectively. Orophayngeal cancer was the commonest site (58.1%) in SCC group whereas oral cavity (86.7%) was commonest for premalignant gruop.(Table 1) Fibrous hyperplasia was the commonest epithelial precursor lesion seen followed by moderate dysplasia.

Table 1
Patient characteristics in study population

	SCC(n=31)	EPL(n=30)	Control(n=30)
Age	55.7 11.4	47.1 14.2	61.5 8.4
M:F	26:5	18:2	21:9
Site of HNCA			
Oral Cavity	4	21	X
Oropharynx	15	1	X
Larynx	5	6	X
Hypopharynx	7	2	X

4.2 Serum Protein and its fractions

We did not observe any abnormality in serum total protein in the two groups and the difference was not statistically significant. Analysing the protein fractions also revealed no deviation from normal. (Table 2).

4.3 Serum Bilirubin

There was mildly elevated total serum bilirubin level in SCC group (1.4mg/dl). The difference in serum direct bilirubin between the groups was statistically very significant ($p<.001$).

Table 2
Liver Function parameters in different study groups

Reference values of parameters	Control (N=30)	Premalignant lesion (N=30)	SCC (N=31)	One way ANOVA Pvalue(<.05)
T PRT (6.6-8.6mgdl-1)	7.4± .5	7.4± .76	7.04± .81	0.0847(ns)
ALB (3.5-5.2mgdl-1)	4.1± .5	4 ±.8	4.05± .6	0.8563(ns)
GLB (2.5-3.5mgdl-1)	3.3± .3	3.2± .7	2.9± .8	0.1637(ns)
BLB(total) (.2-1.3mgdl-1)	1.5± .6	1.2± .5	1.4±.7	0.234(ns)
BLB(direct) (0-.2mgdl-1)	.5 ± .3a,a1	.3± .2 a	.3 ±.1a1	0.009***
BLB(indirect) (0-1.1mgdl-1)	1.03± .6	.94 ±.5	1.15± .59	0.3427(ns)

4.4 Serum aspartate aminotransferase (AST)

The observed levels of serum AST was highly significant with SCC groups showing mean level of 45.7± 20.4 UL. Premalignant group also showed elevated AST but was less as compared to SCC cases (40.9 ±18.9).

4.5 Serum alanine aminotransferase (ALT)

It was also observed that extremely significant increase in serum ALT seen in SCC and premalignant cases (47.7 ±19.8) as compared to Control cases. (p<.0001) .

4.6 Serum gamma glutamyltransferase

The mean serum GGT level was highest in SCC group than in the premalignant group (29.5± 14.6 vs 19.1± 4.8) and the difference of level between the groups was extremely significant.

Table 3
ONE WAY ANOVA analysis of Liver enzymes in different study groups

Reference values of parameters	Control (N=30)	Premalignant lesion (N=30)	SCC (N=31)	One way ANOVA Pvalue(<.05)
AST (10-37UL-1)	27.5± 5.8 a2,b	40.9 ±18.9 a2	45.7± 20.4 b	<.0001***
ALT (10-37UL-1)	24.56±2.9 b1,c	47.7 ±19.8 b1	39.5± 12.3 c	<.0001***
GGT (4-38ul-1)	19.84 ±5.7 c2	19.1± 4.8 c1	29.5± 14.6 c1,c2	<.0001***

All values are the mean±SD. Statistical significance between means was performed using one-way analysis of variance (ANOVA) followed by Duncan as a post ANOVA test (p<0.05). GGT, gamma glutamyltransferase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALB, albumin; BLB, bilurubin; **: p<.01(very significant), *: p<.05(significant), ***:p<.001 (extremely significant),ns: not significant. On comparing the different groups by Tukey-Kramer Multiple Comparisons test, the correspond ding letters indicate the significance level between groups as follows f:p<.05(significant), a,a1,a2:P<.01(very significant), b,b1,c,c1,c,2,d :p<.001 (extremely significant).

Figure 1
Comparison of mean serum AST levels in study groups

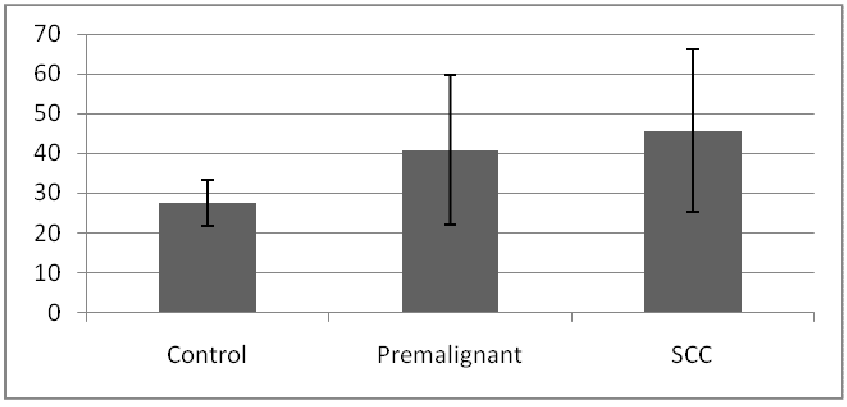

Figure 2
Comparison of mean serum ALT levels in study groups

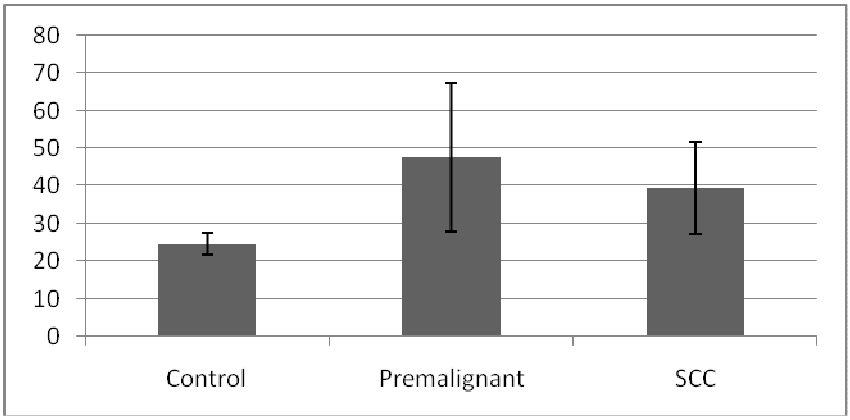

Figure 3
Comparison of mean serum GGT levels in study groups

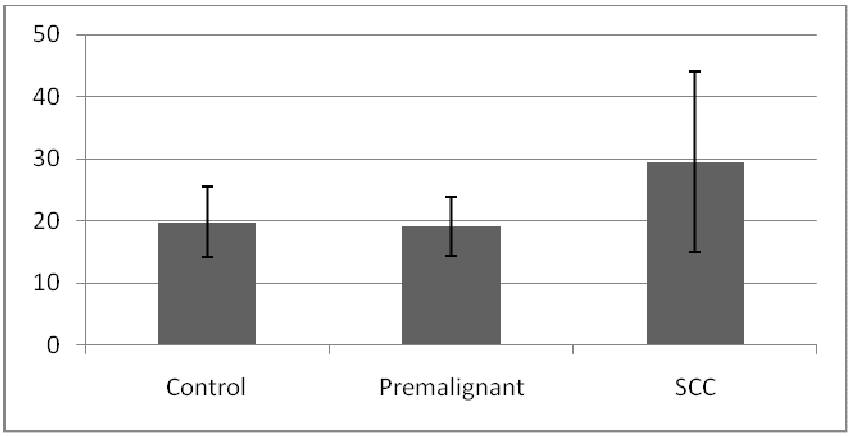

Table 4
Comparative study of serum biochemical parameters in HNSCC and lung cancer

	Present study			Cavusoglu et al (2010)	
Reference values of parameters	Premalignant (N=30)	SCC (N=31)	Control (N= 30)	Lung cancer (N=14)	Control (N=10)
ALB (3.5-5.2mgdl-1)	4 ±.8	4.05± .6	4.1± .5	3.81±0.4	4.50±0.5
BLB (.2-1.3mgdl-1)	1.2± .5	1.4±.7	1.5± .6	0.35±0.1	0.34±0.8
AST (10-37UL-1)	40.9 ±18.9	45.7± 20.4	27.5± 5.8	14.86±3.8	22.20±5.9
ALT (10-37UL-1)	47.7 ±19.8	39.5± 12.3	24.56± 2.9	14.71±5.1	21.90±6.2
GGT (4-38ul-1)	19.1± 4.8	29.5± 14.6	19.84 ±5.7	48.21±30.1	13.30±3.9

All values are Mean±SD. GGT, gamma glutamyltransferase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALB, albumin; BLB, bilurubin

4. DISCUSSION

Oral squamous cell carcinomas (OSCC) have been reported widely worldwide with an incidence of 5%, accounting for 2-4% of all new cancers[2] In India, where the habits of chewing tobacco with betel nut, reverse smoking and heavy alcohol usage are common, its incidence is even higher. In India, HNCA is a major health problem, accounting for 30-40% cancers at all sites with its incidence in North-eastern India at 54.48% [3]. Potentially malignant lesions of oral cavity are relatively common occurring in about 2.5% of the population with a malignant transformation rate of 0.6 to 20% as reported in various studies & locations [4]. Identification of molecular markers in blood would predict the development of cancer in its earliest stage or in precancerous stage. Literature scan reveals very few studies on biochemical parameters and role of AST, ALT and GGT in the diagnosis of malignancies of the head and neck. As till date no such study has been undertaken in this highly prevalent region of North-East India, our study examined the variations of these parameters in HNSCC and premalignant lesions and

their role as biomarkers. The mean levels of total serum protein, albumin and bilirubin did not show any statistically significant change between the control and disease groups. But, statistically significant differences was noted in AST, ALT and GGT levels (Table III). Our results are in agreement with findings of Krover et al and Arun et al [5,6]. Arun et al who studied ninety two HNCA patients undergoing radiotherapy had reported statistically significant mild to moderate progressive rise of AST and ALT levels with increasing severity of the disease and considered it as a prognostic marker [6]. It is difficult to explain the mechanism of changes in serum ALT and AST parameters but it could be related to cell destruction. Although, AST and ALT enzymes are synthesized by hepatocytes and are sensitive and specific enzymes for liver disease [7], chronic alcoholism, tumors of the large intestine[8], hepatocellular carcinoma, tissue injury [9] and liver metastasis in particular [10] also show raised enzymes and are found in other tissues such as kidney, muscle and heart as well. [11,12]. Elevated AST, ALT along with alkaline phosphatase, and lactate dehydrogenase is seen in patients with distant metastasis and liver metastasis in particular [13]. Several studies have reported raised AST and ALT when exposed to radiotherapy [14], different doses of gamma radiation [15] but Kula reported fall in the level when exposed to electromagnetic field [16] This may be due to inactivation of ezymes or alteration of functions [17]. From the present study, we observe that AST and ALT can be considered as a strong candidate for HNCA markers. On examining the serum GGT, there was a statistically significant increase ($p<0.05$) in both the SCC and Premalignant group. Although within the normal range, the serum GGT level (29.5± 14.6) in SCC patients was found to be higher than the control levels (19.84 ±5.7mg/dl). This finding is in agreement with the result of Singh J et al [18] and emphasized the point that GGT activity is induced in human carcinomas [19,20]. GGT, a key enzyme involved in glutathione metabolism, is often highly expressed in human malignancies. Mean serum GGT activity in our SCC group was found to be 29.5± 14.6 as compared to 32.5+- 3.90 IU/L reported in breast cancer [21] and 48.21±30.1 as in lung cancer [14]. GGT is a membrane bound oncofetal glycoprotein, involved as an amino acid transporter by catalytic transfer of gamma-glutamyl groups between peptides or amino acids.[22] Although, its mainly concentrated in proximal convoluted tubules of the kidney , its level have been shown to be altered during carcinogenesis of various cancers. Its high level in SCC and precancer states suggest the release of the membrane bound constituents from different tissues even in carcinoma. Although it is a sensitive indicator for both malignant and non-malignant liver disease, several studies have shown that gamma GT levels are also elevated in other malignant tumours like breast cancer [21, 23] and metastatic renal cell carcinoma [24]. Our study throws light on GGT as a marker for HNCA as well. In fact, Corti etal., [25] in their study considered GGT as a diagnostic and prognostic marker in HNCA.

5. CONCLUSION

The present study shows that AST, ALT are significantly elevated in head neck cancer and precancer conditions. This findings confirms the variation of liver enzymes in HNCA and further larger study need to be undertaken to find the cause or mechanism of this rise in enzymes and establish it as a biomarker in head neck cancer. Moreover, we also conclude that GGT levels rise in HNCA similar to other body malignancies. We propose that determination of serum GGT in premalignant lesions may serve to indicate the malignant transformation of such lesions which are otherwise clinically not possible to predict the changes. However, we suggest further studies on a larger population to establish these enzymes as predictive or prognostic markers. As early detection is the cornerstone of cancer cure, these findings from this highly prevalent region of India will serve as a starting point for further research in this aspect. Liver function test being easily available and affordable, these enzymes can potentially help in the management of head neck cancer.

CONFLICT OF INTEREST

Conflict of interest declared none.

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