Serum Ceruloplasmin and Serum Adenosine Deaminase: Do They have a Role as a Noninvasive Diagnostic and follow Up Adjuncts in Malignant Solid Tumors?

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ABSTRACT

Introduction: Ceruloplasmin is an acute phase reactant, the levels of which fluctuate in response to tissue injuries of various kinds. Adenosine deaminase enzyme on the other hand through purine salvage system is involved in various cellular proliferations. Increased ceruloplasmin and adenosine deaminase activity may be of significance in neoplastic disorders with increased cell turnover. ADA has been evaluated in pleural and ascitic fluids extensively but relatively there are fewer serum ADA studies in malignancy. Serum ceruloplasmin has been assessed as an acute phase reactant in inflammatory conditions but its role in malignancy is still under explored. Aim of this study was to assess the clinical utility of serum ceruloplasmin and serum adenosine deaminase as diagnostic and follow up tools in malignant solid tumors.

Material and methods: Serum concentrations of ceruloplasmin and adenosine deaminase were determined in 100 consecutive cases of solid malignant tumors, at diagnosis and at four weeks after surgical resection of tumor. Samples from 100 healthy young individuals were taken as control.

Results: Serum ceruloplasmin and adenosine deaminase levels were significantly higher in patients compared to the healthy control group. The enzyme parameters showed decrease trend after surgery, however the fall in serum adenosine deaminase level in malignant tumors was found statistically significant.

Conclusion: Serum ceruloplasmin and adenosine deaminase can be used as adjuncts in the diagnosis of various solid malignant tumors. Serum adenosine deaminase may also be useful as a follow up tool in malignant tumors.

Keywords: Ceruloplasmin, Aenosine Deaminase, Solid Malignant Tumors

INTRODUCTION

Malignancy finds its position amongst top five most common causes of death in developing countries and accounts for 9.5% of all deaths. In India there are around 2.5 million cases of malignancy at any given point of time.[1] With ever increasing incidence of cancers year after year, discovery of effective diagnostic, prognostic and follow up markers have been the main stay of cancer research. Several organ specific biochemical markers (e.g. CA-125 and PSA) have already reached from bench to bedside. However, we need generic biochemical markers which constitute simple, inexpensive and routine non invasive investigations in clinical laboratory and have definitive role in screening, diagnosis, prognosis and follow up of malignancies. Serum cerloplasmin (Cp) and Adenosine Deaminase (ADA) are two such markers of inflammation with a potential role in malignancy.

Cp is an acute phase protein, levels of which fluctuate in response to tissue injury of various kinds including trauma, myocardial infarction, acute infections, burns, chronic inflammation (tuberculosis, pyelonephritis, Crohn's disease and rheumatoid arthritis) and malignancy.[2,3] Many theories have been postulated for the cause of rise in serum Cp. In solid tumors, rise in Cp is ascribed to the non-specific inflammation in tissue surrounding the tumor. It could be due to stimulus from inflammatory cytokines or hepatocyte stimulating factors. Malignancy favours angiogenesis which requires copper as an obligatory cofactor. Being a copper containing protein, synthesis of Cp in liver increases, as the availability of copper to liver rises. Cp may also be raised as an antioxidant in the serum of cancer patients to balance out oxidant carcinogens. The tumor cells can also synthesize Cp. Studies have shown significantly increased levels of Cp above the normal before treatment, and the degree of elevation was also related with the tumor stage. High preoperative values correlated with a poor prognosis and elevated postoperative values correlated with residual tumor or recurrences.[4,5]

ADA is an enzyme involved in catabolism of purine bases, responsible for the conversion of adenosine and deoxyadenosine to inosine and deoxyinosine and is involved in the proliferation and differentiation of lymphocytes, particularly the T subtype. ADA activity is found to be high in chronic inflammatory diseases like tuberculosis and rheumatoid arthritis and levels have found to decrease in these diseases with treatment. Perhaps this decrease might reflect the normalization of the altered lymphocyte turnover induced by chronic inflammation. The enzyme is particularly sensitive to stimulation by the growth factors and cytokines during rapid tissue proliferation.[6,7,8] Study of ADA in tissues have shown much higher activities in the tumor tissue than in neighboring mucosa suggesting that the activities

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of purine metabolizing enzymes increase to cope up with accelerated purine metabolism in the cancerous tissue. There is an increase of ADA activity in very rapidly growing malignancies while slow growing well-differentiated tumors do not express pronounced ADA activity.[9,10,11,12]

ADA has been evaluated in pleural and ascitic fluids extensively but relatively there are fewer serum ADA studies. Role of serum Cp and ADA in malignancy is still under explored. This study was directed to find the pattern of serum Cp and ADA in various solid malignancies with an aim to assess the clinical utility of serum Cp and serum ADA as diagnostic and follow up tools in malignant solid tumors.

MATERIAL AND METHODS

This was a prospective study carried out in 100 patients of malignancy and 100 healthy controls. Patients of malignancy were studied from malignant disease treatment center of a tertiary care hospital. Detailed history was taken and clinical evaluation was done for each subject after taking informed consent. Freshly diagnosed consecutive 100 cases of various solid malignancies confirmed by histological examination (FNAC/Biopsies) were included in the study. Paired blood samples were collected in all the cases; at diagnosis and at four weeks after institution of therapy (surgical resection of tumors). Hundred healthy individuals with no significant antecedents of disease or signs and symptoms of any disease were taken as control subjects after informed consent. Single blood samples were collected from healthy controls.

Three ml of blood was collected from antecubital vein by venipunture in labeled plain glass vials and was centrifuged at 3000 rpm for 15 min to separate the serum from cells. The serum was stored in labeled sterile vials at -20ºC until analyzed. Only sera free from hemolysis were used, because erythrocytes contain ADA activity and can falsely increase the ADA results for serum. Both ADA and Cp are stable in serum for at least 24h at 25ºC, 7days at 4ºC and 3 month at -20ºC. Before being used, this serum was allowed to come to room temperature and then mixed by gently inverting the tubes and used for the estimation of Cp and Adenosine deaminase. Serum Adenosine Deaminase was estimated by method of Galanti and Guisti.[13] Serum Cp level by kinetic method based on Ferroxidase activity of Cp.[14] Reference serum values of Cp is 750 ± 250 IU/L and of ADA is < 40 U/L.

STATISTICAL ANALYSIS

Student's unpaired 't' test was used to analyze statistical significance of serum values of Cp and ADA at the point of diagnosis compared to controls in malignancy cases. Student's paired 't' test was applied to analyze the statistical significance of both the biochemical parameters at four weeks after institution of treatment in malignancy cases.

RESULTS

Hundred consecutive cases of solid malignant tumors who registered with malignant disease treatment center, comprising of 22 males and 78 females were between 30 to 76 yrs of age. Malignant cases comprised of 48 cases of breast carcinoma, 14 cases of gastrointestinal cancer, 20 cases of head and neck cancer (oral cavity and larynx), 10 cases of urinary tract (Kidney and urinary bladder), 08 cases of female genital tract (cervix, ovary and endometrium). Controls comprised of healthy males with age ranging between 19 to 52 years. No correlation was found between serum ADA or Cp and age in healthy subjects or patients of either sex (P> 0.05).

Groups	**ADA** Mean Values SD (U/L)	**Cp** Mean Values ± SD (IU/L)
Malignancy cases (at diagnosis)	39.9215.01	1617.76±371.52
Malignancy cases (Post surgery)	31.32±11.57	1557.28±292.3
Healthy controls	18.84± 6.10	644. 90±86.20
SD- standard deviation		

Table-1: Mean ADA & Cp values in cases (paired samples) and controls

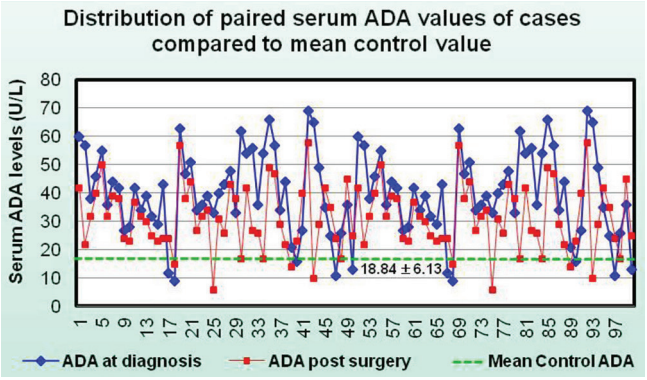

Figure-1: Comparison of pre and post surgery serum ADA levels in malignant cases with reference to controls

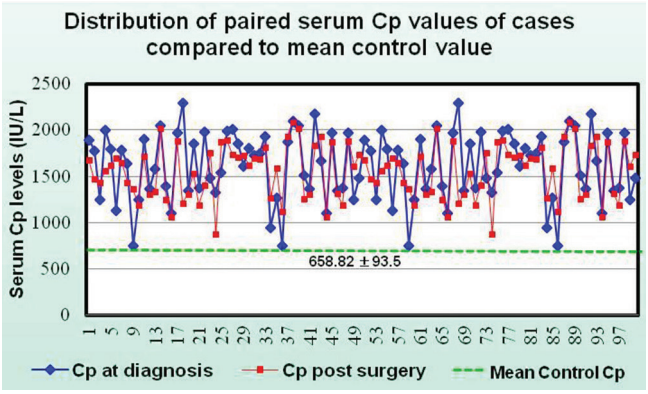

Figure-2: Comparison of pre and post surgery serum Cp levels in malignant cases with reference to controls

Serum ADA and Cp levels in malignancy cases at diagnosis were found to be significantly higher (P<0.001) than those of healthy controls (table 1).Difference in paired mean in case of ADA (Fig 1) was significant (P<0.001) but in case of Cp (Fig 2) difference in paired mean was not statistically significant (P>0.05).

DISCUSSION

Serum Cp showed three fold rise in solid malignant tumors

at the time of diagnosis; the difference was found to be statistically significant ($p<0.001$) in comparison with healthy control subjects (Fig1). Shah et al (2017) got similar findings in their study on oral malignancy.[15]

After surgical removal of tumor, there was decrease in mean Cp levels (Table1) but values remained much above the normal control values ($p>0.05$). This may be attributable to surgery-induced synthesis of acute phase reactant protein or may be due to persistence of residual tumor. Han et al (2017) carried out immunohistochemistry of Cp and indicated its possibility of potential prognostic marker.[16]

Although Cp is an acute phase reactant, this does not detract from its diagnostic usefulness. It would be useful to know whether the increase in serum Cp in patients with advanced tumors, occurs early enough to be useful as a clinical indicator. Whether elevated postoperative values correlated with residual tumor or recurrences needs to be evaluated further. Serum Cp can potentially be used as an adjunct for screening positive diagnosis and monitoring of tumoral disease. The activity of serum ADA at the time of diagnosis was found significantly higher (Fig 2) in patients of solid malignant tumors as compared to controls ($p<0.001$). The mean ADA level of all patients after surgical removal of tumors, remained about two times higher than that of the control subjects, and the difference with initial values (Table1)was found to be statistically significant ($p<0.001$). Kelgandre et al (2016) too in their study got statistically significant increase in serum ADA levels in malignancy.[17]

In our study, the ADA levels of rapidly proliferating tumors like breast carcinomas were higher than those of the head and neck cancers. Rapidly proliferating tumors showed appreciable decrease in levels with the removal of tumor burden. Limitation of our study is that sample sizes of individual cancers were not large enough to apply statistical tests. Further study with larger sample size is required to establish this aspect.

CONCLUSION

To conclude, serum ADA and Cp can be used as adjuncts in the diagnosis of malignancies. Serum ADA can potentially be used as follow up tool for monitoring the response to treatment in malignant tumors. Role of Cp in malignancies in monitoring the response to treatment is equivocal. Not much has been done specifically involving serum ADA and Cp in cancers. Further large scale studies with individual cancers need to be explored.

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