

A STUDY OF SERUM ZINC, COPPER, CALCIUM AND MAGNESIUM CONCENTRATIONS IN PATIENTS OF COLORECTAL CARCINOMA AND HEALTHY CONTROLS IN ASSAM MEDICAL COLLEGE AND HOSPITAL

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Abstract

Background: Trace element deficiency or excess is implicated in the development or progression in some cancers including colorectal carcinoma.^[1,2] By characterizing the role of serum levels of trace elements namely zinc, copper, calcium and magnesium it can be inferred that these are responsible for the rising trend of colorectal carcinoma in North East India.^[3] **Materials and Methods:** A hospital based case control study including confirmed cases of CRC (colorectal carcinoma) attending outpatient department of Surgery with equal number of adequately matched healthy controls were enrolled from June 2017- May 2018. Serum estimation of Calcium, Zinc, Copper, Magnesium were done and analysed. **Result:** Serum copper concentration was found to be significantly higher in patients suffering from CRC than normal controls. Mean serum levels of Zinc, magnesium and calcium were significantly lower in patients of CRC. **Conclusion:** The present study point towards association of Zinc, Copper, Calcium, Magnesium imbalance in the progression of CRC.

INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer worldwide with an estimated one million new cases and a half million deaths each year.^[1,2] Colorectal cancers is thought to result from a complex interaction between inherited susceptibility and environmental factors, as indicated by genetics and experimental and epidemiological studies.^[3,4,5] According to observational studies that had been performed, development of Colorectal carcinoma may be related to environmental factors such as high calorie intake, high consumption of red meat, high consumption of saturated fat, obesity, cigarette smoking and a sedentary lifestyle.^[6] Trace element deficiency or excess is implicated in the development or progression in some cancers.^[7,8] Copper(Cu) is a redox active transition metal that can participate in single electron reaction and catalyze formation of free radicals. Cu has been suggested to play an important role in several disorders and normal immune function.^[9,10] Therefore, serum Cu determinations may be important while assessing colon cancer patients. Zinc(Zn) is a component of many DNA repair proteins. It is also involved in a variety of general

cellular functions such as cell signal transduction, transcription and replication.^[11] Zn has important role in prevention of cancer formation by stabilizing the structure of deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and ribosomes. Magnesium (Mg) is an essential mineral which plays a role in a number of cellular metabolic reactions. It plays a role in tumor biology via the regulation of oxidative stress, carcinogenesis, tumor progression, and angiogenesis.^[12,13,14,15] Calcium (Ca) has been shown to play a primary role in the development of cell injury in different pathological states of vital organs. Disturbances of calcium homeostasis may be induced in three different sub cellular compartments including cytoplasm, mitochondria or the endoplasmic reticulum.^[16] There is an increasing trend of cases of colorectal carcinoma attending Assam Medical College and Hospital as seen in records which parallels with rising trend of this disease in the community. Typical Assamese diet which consists of high animal proteins, less of dietary fibres and fruits, high consumption of alcohol and smoking predispose the population towards high risk for colorectal carcinoma. Combined with this epidemiological predisposition is the malady of poverty which delays early contact

of population with health care system resulting in advanced stage of carcinoma and dismal prognosis. To address this issue we need to fully delineate the risk factors and possible associations of various factors present in environment and lifestyle. Hence, present study shall be useful for characterizing the role of trace elements namely zinc, copper, calcium and magnesium which may be responsible for the rising trend of colorectal carcinoma in past few years. To my knowledge no study in the past has been conducted to estimate quantitatively the trace elements concentration in patients of colorectal carcinoma in the North-Eastern region.

Aim

To assess serum zinc, copper, calcium and magnesium concentration in patients with colorectal carcinoma and healthy controls.

Objectives

1. To estimate serum levels of copper, zinc, calcium, magnesium in colorectal cancer.
2. To compare levels of serum copper, zinc, calcium, magnesium between patients of colorectal carcinoma and healthy control.

MATERIALS AND METHODS

The study was carried out on diagnosed cases of colorectal carcinoma (CRC) who attended and/or was admitted in the department of Surgery, Assam Medical College and Hospital, Dibrugarh. It was a one-year hospital based case control study

Place of Study

Department of Biochemistry and Advanced Clinical Biochemistry Laboratory & Department of Surgery, Assam Medical College and Hospital; Dibrugarh.

Study Group

Cases

52 histopathologically confirmed cases of colorectal carcinoma attending the Surgery department of Assam Medical College and Hospital were enrolled for a period of one year. CONTROLS: Equal numbers (52) of apparently healthy, age and sex matched individuals and identical socioeconomic status, without any evidence of disease were included in the study.

Criteria for Selection of Cases

Inclusion Criteria

1. Patients with colorectal carcinoma at any stage of cancer.
2. Age \geq 30 years.

Exclusion Criteria

1. Age less than 30 years
2. Under chemotherapy or Chemotherapy course finished
3. Patients on zinc/ copper/calcium/magnesium supplementation
4. Post-operative follows up patients.

Collection of Blood Sample

About 10 ml. of blood was collected preoperatively of cases and control from antecubital vein after proper aseptic and antiseptic measures in sterile vial for estimation of Cu, Zn, Mg & Cu. Sample collected was allowed to clot and then centrifuged at 3000 rpm for 10 minutes. Then the supernatant serum was taken and the following estimations performed using semiautoanalyzer, MICROLAB 300.

Estimation of serum Zinc by colorimetric method.^[17]

Principle

Zinc in an alkaline medium reacts with Nitro-PAPS to form a purple coloured complex. Intensity of the complex formed is directly proportional to the amount of Zinc present in the sample.

Zinc + Nitro-PAPS ----->
purple coloured complex
Alkaline Medium

Normal Reference Values

Serum: 60-120 μ g/dL

Estimation of serum Copper by colorimetric method.^[18]

Principle

Copper released from ceruloplasmin, in an acidic medium, reacts with Di-Br-PAESA to form a coloured complex. Intensity of the complex formed is directly proportional to the amount of Copper present in the sample.

Copper + Di-Br-PAESA ----->
Coloured complex
Acidic Medium

Normal Reference Range

Serum copper Male: 80-140 μ g/dl
Female: 80-155 μ g/dl

Estimation of serum Calcium by Arsenazo III method.^[19]

Principle

Calcium combines specifically with Arsenazo III at a neutral pH to form a blue purple coloured complex. Intensity of the colour formed is directly proportional to the amount of calcium present in sample.

Calcium + Arsenazo III \square Blue
purple coloured complex

Normal Reference Values

Serum 8.5-10.1 mg/dl

Estimation of serum Magnesium by Calmagite method.^[20]

Principle

Magnesium combines with Calmagite in an alkaline medium to form a red coloured complex. Interference of calcium and proteins is eliminated by the addition of specific chelating agents and detergents. Intensity of the colour formed is directly proportional to the amount of magnesium present in the sample.

Magnesium + Calmagite \longrightarrow Red coloured complex
Alkaline medium

Normal Reference Values

Serum 1.8-2.4 mg/dl.

RESULTS

Mean age of patients presenting with Colorectal Carcinoma (CRC) in the present study is 45.5 years. Maximum number (n=16) (30.8%) of patients belong to age group 40-50 years with younger age group of 35-40 years forming second largest group of patients (n=13)(25%). This might reflect an epidemiological shift in CRC occurring during younger age or early detection of cases owing to robust health services. (n=9) (17.3%) of cases are detected in geriatric age group of 60-70 years. In elderly geriatrics of age > 70 years' number of study subjects has been (n=5) (9.6%). Appropriate healthy age matched controls have been selected for comparison with cases.

Sex Distribution

Most of the patients suffering from CRC are males (n=29) (55.8%) while females (n=23) (44.2%). The ratio of M: F is 1.26. Controls have been age and sex matched with respect to cases. In the present study biases of age and sex between cases and controls have been diligently addressed by selecting controls from healthy population almost of same age and sex.

Clinical Presentation

Pallor with weakness, being the most common presenting sign is present in 71.2% of patients. 59.6% of cases presented with pain abdomen. The pain abdomen was either crampy or constant localised to the site of the lesion. While 57.7% of cases reported bleeding per rectum of fresh blood streaked or admixed with stool. This being the second most common symptom of cases suffering from CRC. This rectal bleeding was during defecation. Similarly altered bowel habits was seen in 53.8% of cases of CRC. Repeated unsuccessful attempts to defecate was made by cases with passage of only flatus mostly and loose stools at times. This was also associated with spurious early morning diarrhoea with blood and mucus.

However, 48.1% of cases had acute presentation of large bowel obstruction with complaints of

obstipation, nausea/vomiting, distended abdomen, pain abdomen often with signs of severe dehydration due to intravascular fluid sequestration and loss (in vomitus).

Site Distribution of Growth

The commonest site of CRC in present study is rectum (n=24) (46.2%) constituting almost half of the cases. The second most common site is sigmoid /recto sigmoid junction with (n=12) (23.1%) of cases. Rectum and sigmoid constitute almost two-third of CRC (n=36) (69.3%). The third most common site is cecum/and ascending colon (n=8) (15.4%) followed by transverse colon with hepatic/splenic flexure (n=4) (7.7%) and descending colon (n=4) (7.7%). Occurrence of Left sided (90%) malignancy is far greater than right sided (10%) in present study.

TNM Stage

In present study, all the patients were staged based on AJCC 7th edition TNM classification using CECT abdomen, CT chest, and histopathological report of resected specimen of CRC. 2(3.84%) patients belonged to stage I, 7(13.44%) patients of stage II, 27 (51.92%) patients were of stage III while 16(30.72%) patients had distant metastasis or regional adjacent organ infiltration (stage IV).

ZINC

Mean Serum Zinc levels in cases is 44.9 ± 32.9 $\mu\text{g/dL}$. Minimum level is 13.80 $\mu\text{g/dL}$ while maximum being 158.9 $\mu\text{g/dL}$ thus range being spread over 145.10 $\mu\text{g/dL}$. Median and mode values are respectively 32.55 $\mu\text{g/dL}$ & 21.4 $\mu\text{g/dL}$. The normal range of Zinc standardised for regional population is $60-120$ $\mu\text{g/dL}$. Mean Serum Zinc levels in controls is 97.70 ± 24.08 $\mu\text{g/dL}$. Minimum level is 70.00 $\mu\text{g/dL}$ while maximum being 196.50 $\mu\text{g/dL}$ thus range being spread over 126.50 $\mu\text{g/dL}$. In present study, mean serum Zinc levels of cases are lower than the normal range for the population. Also when compared to serum level of Zinc in controls, it is found to be significantly lower ($p < .05$) (Table 1)

Table 1: Analysis of Variance Test (Anova) To Test Level of Significance of Difference of Cases and Controls in Serum Zinc Levels. **P<.05 Indicating Significant Difference

ANOVA					
Anova Analysis	Sum Of Squares	Df	Mean Square	F	P Value
Between Groups	72351.900	1	72351.900	86.736	.001**
Within Groups	85084.945	102	834.166		
Total	157436.845	103			

Site of Origin of Colorectal Carcinoma and Serum Zinc Concentration

Maximum mean serum zinc levels are found in sigmoid colon cancers 49.22 ± 37.10 $\mu\text{g/dL}$. Lowest mean values are found in cecum/ ascending colon malignancies 34.82 ± 24.50 $\mu\text{g/dL}$. The patient's serum zinc level analysis do not demonstrate significant difference with respect to different sites of origin of colorectal carcinomas ($p = 0.80$).

TNM Stages and Serum Zinc Concentration Analysis

Maximum mean serum zinc levels were recorded in stage II patients 72 ± 56.26 $\mu\text{g/dL}$ while lowest levels were found in stage III cancers 37.88 ± 24.62 $\mu\text{g/dL}$. However, the difference in serum Zinc levels compared among various TNM stages is not statistically significant ($p = 0.106$)

Copper Mean Serum Copper levels in cases is 180.14 ± 104.12 $\mu\text{g/dL}$. Minimum level is 34.80 $\mu\text{g/dL}$ while maximum being 577.90 $\mu\text{g/dL}$ thus range being spread over 543.10 $\mu\text{g/dL}$. Median and mode values are

respectively 163µg/dL & 164.80µg/dL. Mean serum Copper levels in controls is 90.83± 7.79 µg/dL. Minimum level is 80.1µg/dL while maximum being 120.1 µg/dL thus range being spread over 40.0 µg/dL. The normal range of Copper standardised for regional population is 80-140 µg/dL. Thus we find that mean serum copper levels of cases are higher than the normal range [Table 2].

Table 2: Analysis of Variance Test (Anova) To Test Level of Significance of Difference of Cases and Controls in Serum Copper Levels. **P<.05 Indicating Significant Difference

Anova Analysis	Sum Of Squares	Df	Mean Square	F	P Value
Between Groups	207345.670	1	207345.670	38.039	.001**
Within Groups	555987.204	102	5450.855		
Total	763332.874	103			

Site of Origin of Colorectal Carcinoma and Serum Copper Levels

Maximum mean serum copper levels are found in descending colon cancers 340.0±178.4 µg/dL. Lowest mean values are found in sigmoid colon and rectal malignancies 148.04±66.79 µg/dL and 149.75± 74.44 µg/dL respectively. Hence right sided CRC demonstrate significantly higher (p=.003) levels of serum copper compared to rectosigmoid CRC.

TNM Stages and Serum Copper Concentration Analysis

Maximum mean serum copper levels were recorded in stage IV patients 229±0.05 mg/dL while lowest levels were found in metastatic stage I cancers 153.4±10.96 mg/dL. However, the difference in serum copper levels compared among various TNM stages is not statistically significant (p=0.142)

Calcium

Mean Serum calcium levels in cases is 8.05±0.82 mg/dL. Minimum level is 6.70 mg/dL while maximum being 10.1 mg/dL thus range being spread over 3.4 mg/dL. Median and mode values are respectively 7.85 mg/dL & 7.20 mg/dL. The Mean Serum Calcium levels in controls in present study is 8.74± 0.62 mg/dL. Minimum level is 6.90 mg/dL while maximum being 9.9 mg/dL thus range being spread over 3.0 mg/dL. The normal range of Calcium standardised for regional population is 8.5 – 10.1 mg/dL. Thus we find that mean serum calcium levels of cases is lower than the normal range [Table 3].

Table 3: Analysis of Variance Test (Anova) To Test Level of Significance of Difference of Cases and Controls in Serum Calcium Levels. **P<.05 Indicating Significant Difference

ANOVA					
Serum Calcium	Sum Of Squares	Degree Of Freedom	Mean Square	F	P Value
Between Groups	2.222	1	2.222	4.158	.044**
Within Groups	54.498	102	.534		
Total	56.720	103			

Site of Origin of Colorectal Carcinoma and Serum Calcium Levels

Maximum mean serum calcium levels are found in descending colon cancers of 8.60±0.35 mg/dL while lowest values are found in sigmoid cancers of 7.83±0.93 mg/dL. p value=.563 i.e. the difference between serum calcium levels of various sites of origin of CRC are not statistically significant

NM Stage and Serum Calcium Levels

Maximum patients (n=27) (51.9%) belonged to stage III with mean serum calcium levels 8.14±0.94 mg/dL. Maximum serum calcium levels were recorded in stage II patients of 8.27±0.80 mg/dL while lowest levels were found in metastatic stage IV cancers 7.80±0.61 mg/dL. However, the difference in serum calcium levels based on TNM staging is not statistically significant. (p=0.514)

Magnesium

Mean Serum Magnesium levels in cases is 1.92± 0.27 mg/dL. Minimum level is 1.1 mg/dL while maximum being 2.40 mg/dL thus range being spread over 1.30 mg/dL. Median and mode values are respectively 1.95 mg/dL & 2.00 mg/dL. Mean serum Magnesium levels in controls is 2.07± 0.24 mg/dL. Minimum level is 1.7 mg/dL while maximum being 2.70 mg/dL thus range being spread over 1.00 mg/dL. The normal range of Magnesium standardised for regional population is 1.8-2.4 mg/dL. Thus we find that mean serum magnesium levels of cases lies within the normal range [Table 4].

Table 4: Analysis of Variance Test (Anova) To Test Level of Significance of Difference of Cases and Controls in Serum Magnesium Levels. **P<.05 Indicating Significant Difference

Anova Analysis	Sum Of Squares	Df	Mean Square	F	P Value
Between Groups	.527	1	.527	7.976	.006**
Within Groups	6.733	102	.066		
Total	7.260	103			

Site of Origin of Colorectal Carcinoma and Serum Magnesium Levels

Maximum mean serum magnesium levels are found in sigmoid colon cancers of 2.03 ± 0.34 mg/dL. Lowest mean values are found in transverse and descending colon malignancies 1.95 ± 0.17 mg/dL and 1.95 ± 0.12 mg/dL respectively. However, the difference between serum magnesium levels compared to various sites of origin of CRC are not statistically significant. ($p=0.626$)

TNM Stages and Serum Magnesium Concentration Analysis

Maximum serum magnesium levels were recorded in stage III patients of 1.98 ± 0.25 mg/dL while lowest levels were found in metastatic stage II cancers 1.84 ± 0.26 mg/dL. However, the difference in serum magnesium levels compared among various TNM stages is not statistically significant. ($p=0.537$).

CONCLUSION

The present study showed following inferences and conclusions

- Majority of patients with colorectal carcinoma belonged to middle years age group of 40-50 years: a shift seen from earlier trends which showed most of the cases occurring in elderly age group. This might reflect an epidemiological shift in CRC occurring during younger age or early detection of cases owing to robust health service/ health awareness.
- Gender wise, the ratio is slightly tilted towards males with Male: Female = 55:45.
- Mean serum zinc levels in patients were significantly lower ($p < .001$, extremely statistically significant) than normal controls. The decreased levels in serum zinc in colon cancer patients may be due to the mobilization of circulating Zn to the colon cancer tissue and its involvement in the antioxidant defence since the patients might be under higher oxidative stress.
- Serum copper concentration was found to be significantly higher in patients suffering from CRC than normal controls thus highlighting role of copper in colorectal carcinoma. Further research is needed to address the issue whether elevated copper levels have causative role in colorectal carcinoma oncogenesis, progression, adenocarcinoma differentiation or just an association.
- Though in present study, serum copper levels in right sided CRC were higher ($p = .003$, extremely significant) than in recto sigmoid CRC. Interestingly, this kind of location specific difference in trace element serum levels has not been demonstrated for Zinc, Magnesium and Calcium. Serum Calcium concentration in cases with colorectal carcinoma was found to be significantly lower ($p < .05$) than serum levels in controls. In published literature, how exactly serum calcium influences carcinogenesis remains indefinite
- Mean serum concentrations of Magnesium in patients with colorectal carcinoma is significantly lower ($p < .05$) than serum levels in controls. It plays a role in tumor biology via the regulation of oxidative stress, carcinogenesis, tumor progression, and angiogenesis according to published literature.

Therefore, trace element deficiency or excess may be implicated in the development, site location or progression of colon cancer. The present study point towards a role of trace element imbalance, especially Zinc, Copper, Calcium, Magnesium in the colorectal cancer. However, is it a direct cause effect relationship or a consequence remains a big question. Further studies are warranted to determine whether changes in serum trace elements may represent an independent risk factor for the development of colorectal cancer and in turn a possible target for preventive intervention.

Declaration

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Ethics Approval

Approved by institutional ethical committee.

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