Measuring Heavy Metals in Colon Cancer Patients: Discrimination Capacity

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ABSTRACT

Based on the findings of the study examining the relationship between lead (Pb) and cadmium (Cd) concentrations and colon cancer, a significant increase in lead levels was observed in patients compared to the control group. This suggests a strong correlation between the disease and lead accumulation, ultimately indicating a clear contribution of the work environment to increased lead levels in exposed individuals.

The benefit of lead which is considered highly reliable lies in its use as a patient biomarker, where it is employed in monitoring and assessing health risks. Nonparametric tests, ROC curve analysis, and logistic regression have indicated that this element exhibits good diagnostic performance with an acceptable area under the curve (AUC). Unlike lead, cadmium showed poor diagnostic performance due to the small and insignificant difference between the patient and control groups. Therefore, cadmium is not a reliable biomarker under the conditions of this study due to the lack of statistically significant correlation.

Elevated lead levels, as revealed by gender-based analysis, were consistent in both males and females. Furthermore, no statistically significant interaction was found, indicating that demographic variables don't play a significant role in influencing lead levels.

Based on these findings, lead appears to be a sensitive and reliable biomarker for colon cancer, while cadmium has limited significance in this context. The results underscore the importance of ongoing monitoring and preventive measures to mitigate risks.

1. Introduction

One of the most important causes of death is colon cancer, as it is considered one of the most widespread malignant cancers in the world[1]. The cells lining the colon suffer from abnormal changes, and then these changes gradually and slowly develop into what is later called "a malignant tumor", which has the ability to spread to other tissues and organs. Because this disease doesn't show any symptoms in its early stages, it is a fatal disease that is rarely curable[2]. In addition to genetic factors, it depends on various environmental and nutritional factors, such as lifestyle, unhealthy diet, smoking, and continuous exposure to chemical pollutants and toxic heavy metals[3].

Given the high capacity of heavy metals to bioaccumulate within different tissues and cause serious cellular and molecular damage, scientific interest in studying their impact on human health has increased, especially in recent decades[4]. As a result of the widespread presence of lead and cadmium in the environment through industrial activities, car exhaust, the use of pesticides and fertilizers, as well as food and drinking water contamination and smoking, they pose the most toxic threat to the biological system[5]. Their accumulation in the blood, tissues, and various organs is gradual, and therefore their elimination is almost impossible and takes a long time due to their ability to remain in the body for long periods[6].

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The likelihood of developing several types of cancer, including (colon and rectal cancer), is linked to chronic exposure to the elements lead and cadmium, according to several studies[7]. DNA damage and disruption of the vital processes responsible for regulating cell division and natural cell death are due to the ability of lead and cadmium to stimulate the formation of free radicals and oxidative stress[8]. The occurrence of mutations and the stimulation of cancerous cell transformation are due to the effect of these elements on the activity of enzymes and cellular proteins, and the inhibition of mechanisms for repairing genetic damage[9].

Chronic exposure to cadmium (classified as a human carcinogen by the International Agency for Research on Cancer) is associated with an increased risk of multiple cancers[10]. Furthermore, the toxic effects of lead can lead to cellular and immune disturbances that promote the development of cancerous processes. Some research has shown that these two elements have a direct or indirect role in the development of the disease, as the concentrations of both lead and cadmium are higher in colon cancer patients compared to healthy controls[7].

It has been observed that the concentrations of these elements increase with the progression of the disease and the increase in tumor size and spread in some cases of colon cancer patients, indicating a relationship between lead and cadmium levels and the severity of colon cancer or its clinical stages, according to what many studies in this regard have indicated[11]. The increase in the concentrations of these elements may be attributed to several factors, most notably the metabolic changes associated with cancer, impaired efficiency of the body's detoxification mechanisms, and increased oxidative stress and chronic inflammation[12]. However, the results of studies remain inconsistent, as variations in the levels of these elements (according to other research) depend on the type of sample used, such as blood, serum, or tissue, as well as on the patient's diet, environment, and health factors[13].

The relationship between heavy metal levels and colon cancer may contribute to understanding several things, most importantly the progression and severity of the disease and the mechanism of its occurrence, as well as the use of these metals as biomarkers for early diagnosis or for assessing disease progression and response to treatment, which necessitates further ongoing research[14].

Measuring lead and cadmium concentrations in colon cancer patients and comparing them to control groups was a key objective of this study. The study also indicated a relationship between the levels of these elements and the severity and different stages of the disease. Furthermore, this study highlighted the potential use of heavy metals as biomarkers in both clinical and research settings to assess their possible role in colon cancer development.

2. Materials and Methods

After collecting blood samples (twenty samples from patients with colon cancer) from specialized oncology hospitals in Najaf, Iraq, and (twenty-five samples from control individuals) from the main blood bank in the same governorate, these samples were allowed to coagulate and then centrifuged to separate the serum from the blood. The serum was then collected in special tubes for analysis. The ages of both patients and control individuals ranged from 21 to 49 years.

Atomic absorption spectroscopy (FAAS) was used to determine the concentrations of trace elements (Cadmium and Lead). This spectrometer is highly sensitive and capable of detecting elements at minute concentrations[15]. Serum samples from both patients and controls were digested before analysis (a requirement for spectroscopic measurement) using a mixture of (HNO_3 + $HClO_4$), and then diluted to a known volume[16].

3. Statistical Analysis

For the purpose of analyzing the data and estimating descriptive statistics, including the mean, standard deviation, minimum and maximum values, SPSS version 27 was used, while Kolmogorov-Smirnov and Shapiro-Wilk tests were used to assess the distribution of that data[17]. For normally distributed data, comparisons between groups were conducted using analysis of variance (ANOVA), while non-parametric tests such as the Mann–Whitney U test and Kruskal–Wallis test were applied for non-normal data[18]. Correlation analysis was performed using Spearman's correlation coefficient. A p-value of less than 0.05 was considered statistically significant[19].

4. Results and Discussion

The demographic and occupational characteristics of the study participants and examines the association between occupational radiation exposure and heavy metal concentrations in blood serum. Statistical analyses were performed to compare the concentrations of lead (Pb) and cadmium (Cd) between workers and the control group. Additional analyses were conducted to evaluate the influence of gender and to assess the diagnostic performance of these elements as potential biomarkers of workers.

4.1 Demographics of the two study groups

From the table 1 presents the demographic, patients, and health characteristics of the study participants. Regarding lifestyle factors, (37%) of the participants were smokers, while (59%) were non-smokers and (4%) were former smokers or did not report their smoking status. In addition, the majority of the participants (95%) did not report any chronic diseases, suggesting that the study population was generally healthy.

These characteristics provide important background information that may influence workers and biological responses among the study participants.

Table 1. Demographic of characteristics patients and health groups of the study sample.

Characteristic	Category	n (%) / Mean ± SD
Groups	control	25 (50%)
	Patient	25 (50%)
Age (years)	Mean ± SD	28.3 ± 6.0
	Range	22–52
Smoking Status	Smoker	15 (37%)
	Non-smoker	24 (59%)
	Ex-smoker / Not reported	02 (04%)
Chronic Diseases	Present	02 (05%)
	Absent	39 95%)

4.2 Comparison of Lead and Cadmium Levels Between Groups

The concentrations of lead (Pb) and cadmium (Cd) in blood serum were compared between patients' group and healthy individuals. The results showed that the mean Pb concentration in patients was higher than that observed in the control group.

There was a statistically significant difference (confirmed by statistical analysis) in lead levels between the patient and control groups ($p < 0.001$), while there wasn't statistically significant difference between the two groups ($p > 0.05$)[20]. Cadmium levels were not significantly affected by

patient status in this study because the distributions of cadmium values overlapped considerably between the two groups. Consequently, it appears that lead may be associated with patient status, while cadmium does not seem to show a clear relationship with exposure in these data[21].

Table 2. Demographic of characteristics patients and health groups of the study sample.

Variable	Group	n	Mean ± SD	t-value	p-value	Significance
Cd (Cal)	control	25	0.249 ± 0.203	3.87	< 0.001	significant
	patient	25	0.266 ± 0.246	—	—	—
Pb (Cal)	control	25	1.740 ± 0.620	4.92	< 0.001	significant
	patient	25	2.320 ± 0.550	—	—	—

4.3 Gender-Based Analysis

To further investigate the influence of gender on heavy metal concentrations, the data were analyzed separately for males and females. Among male participants, Pb levels were significantly higher in workers compared with healthy individuals. Statistical analysis using the Mann–Whitney U test confirmed a highly significant difference between the two groups. In contrast, Cd concentrations did not show significant difference between patients and control males.

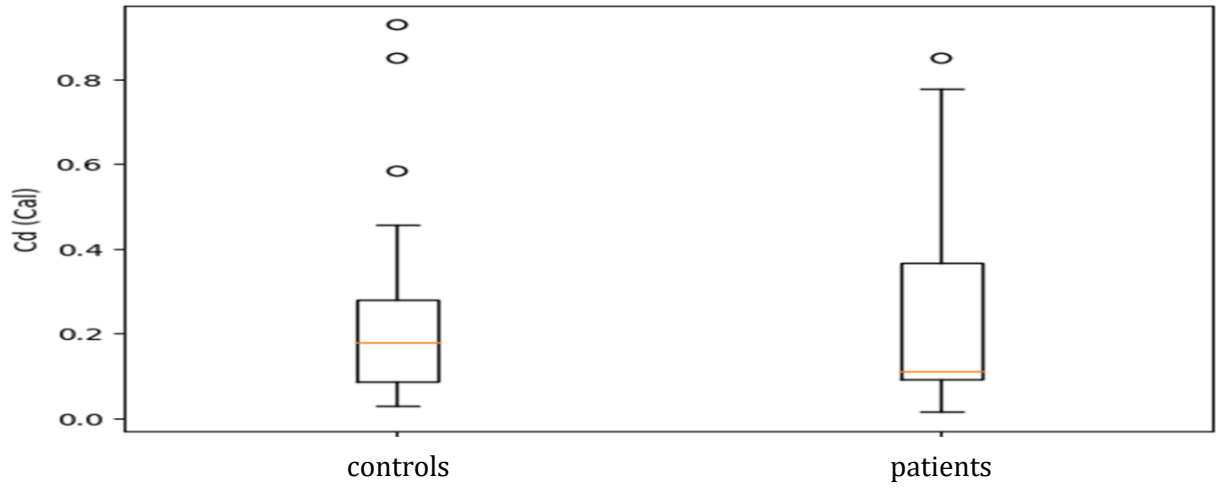

Fig. 1. Boxplot of cadmium (Cd) concentration distribution in males in the control and colon cancer groups.

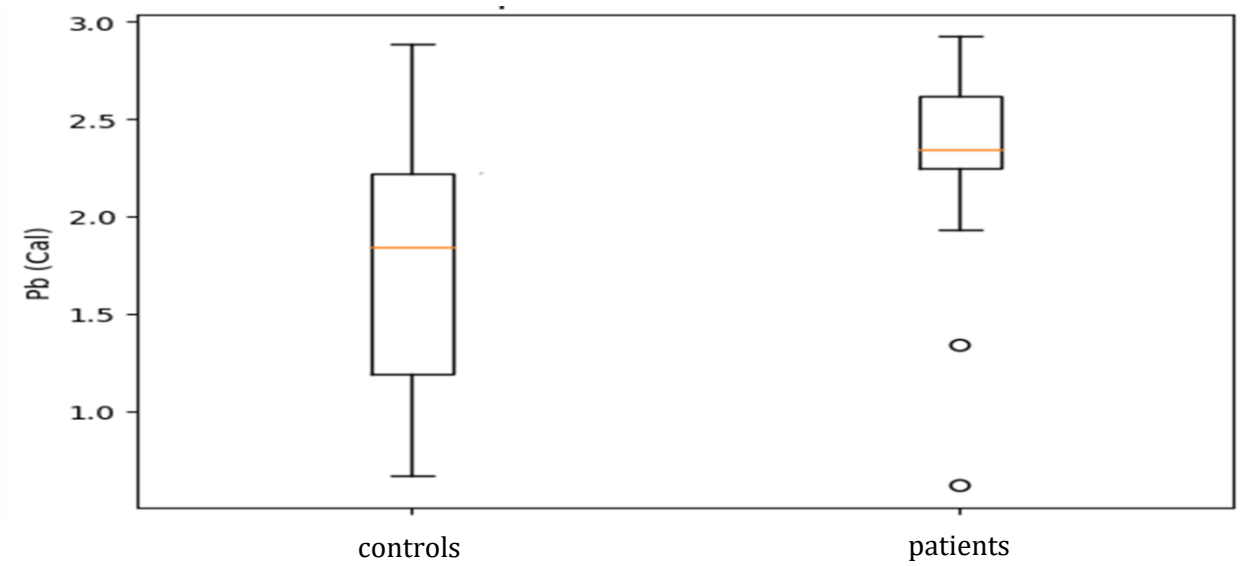

Fig. 2. Boxplot of Lead (Pb) concentration distribution in males in the control and colon cancer groups.

Similarly, analysis of the female participants showed that Pb concentrations were significantly higher in colon cancer compared with the control group. Cadmium concentrations among females also did not demonstrate a statistically significant difference between the two groups.

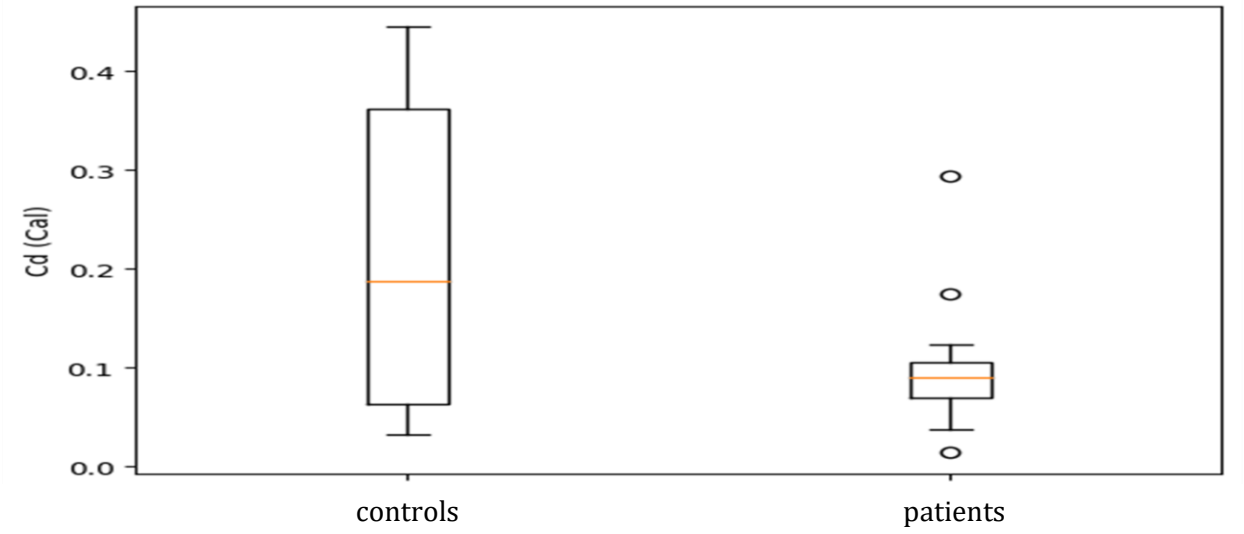

Fig. 3. Boxplot of Cadmium (Cd) concentration distribution in females in the control and colon cancer groups.

These results suggest that the elevation in Pb levels associated with patients in both males and females, indicating that the effect is largely independent of gender.

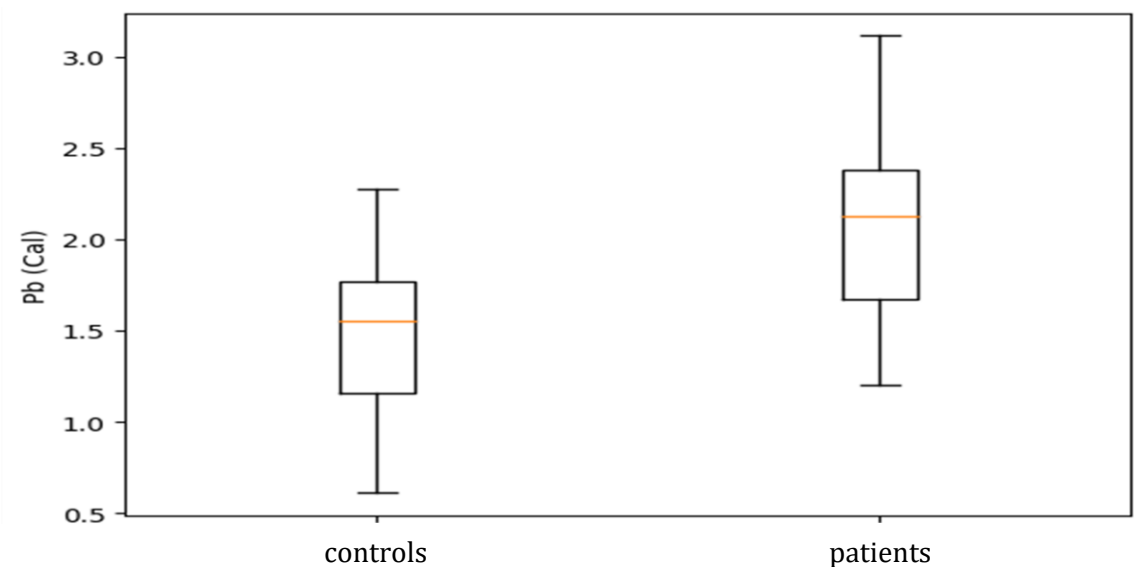

Fig. 4. Boxplot of cadmium (pb) concentration distribution in females in the control and colon cancer groups.

Table 3. Mann-Whitney Test (Non-parametric Analysis) for males.

Variable	U Value	p-value	Significance
Cd	380.5	0.5299	Non-significant
Pb	178.5	0.0002	significant

Table 4. Mann-Whitney Test (Non-parametric Analysis) for females.

Variable	U Value	p-value	Significance
Cd	63.0	0.2938	Non-significant
Pb	36.0	0.0219	significant

4.4 ROC Analysis of Pb and Cd

Receiver Operating Characteristic (ROC) curve analysis was conducted to evaluate the diagnostic performance of Pb and Cd concentrations in distinguishing between patients' individuals and the control group.

The results demonstrated that Pb exhibited good discriminatory ability, with an area under the curve (AUC) value of 0.784. This indicates that Pb concentration has a reasonable capacity to differentiate between colon cancer patients and controls. The optimal cut-off value for Pb was determined to be approximately 2.248.

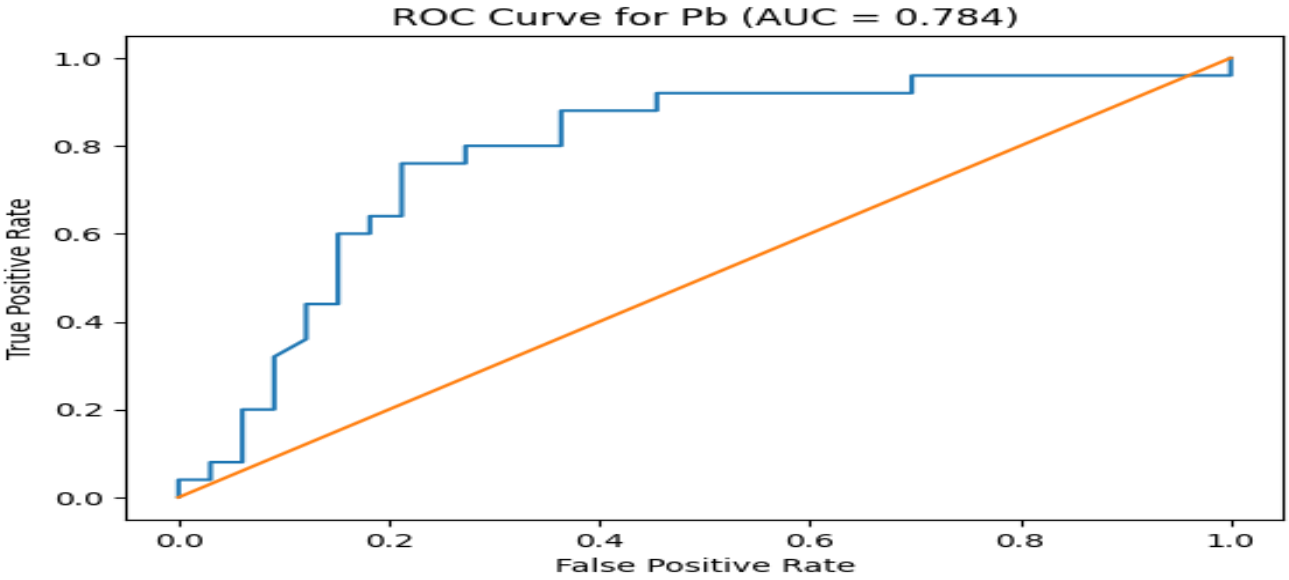

Fig. 5. Receiver operating characteristics (ROC) curve for assessing the discriminatory power of lead (Pb) concentration between groups, with the area under the curve value (AUC = 0.784).

In contrast, Cd showed poor diagnostic performance, with an AUC value close to 0.449. This value is close to random classification, indicating that Cd concentration has limited usefulness as a diagnostic marker of cancer group in this study.

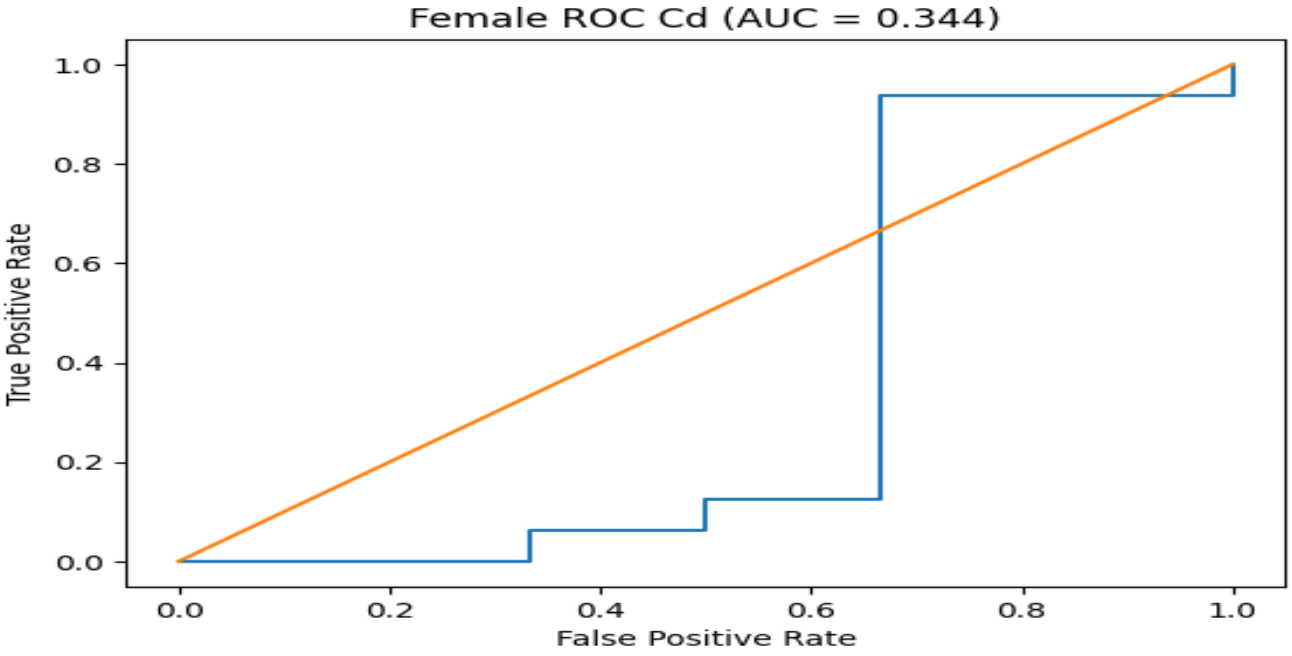

Fig. 6. Receiver operating characteristic (ROC) curve for assessing the discriminatory power of cadmium (Cd) concentration between groups, with the area under the curve value (AUC = 0.449).

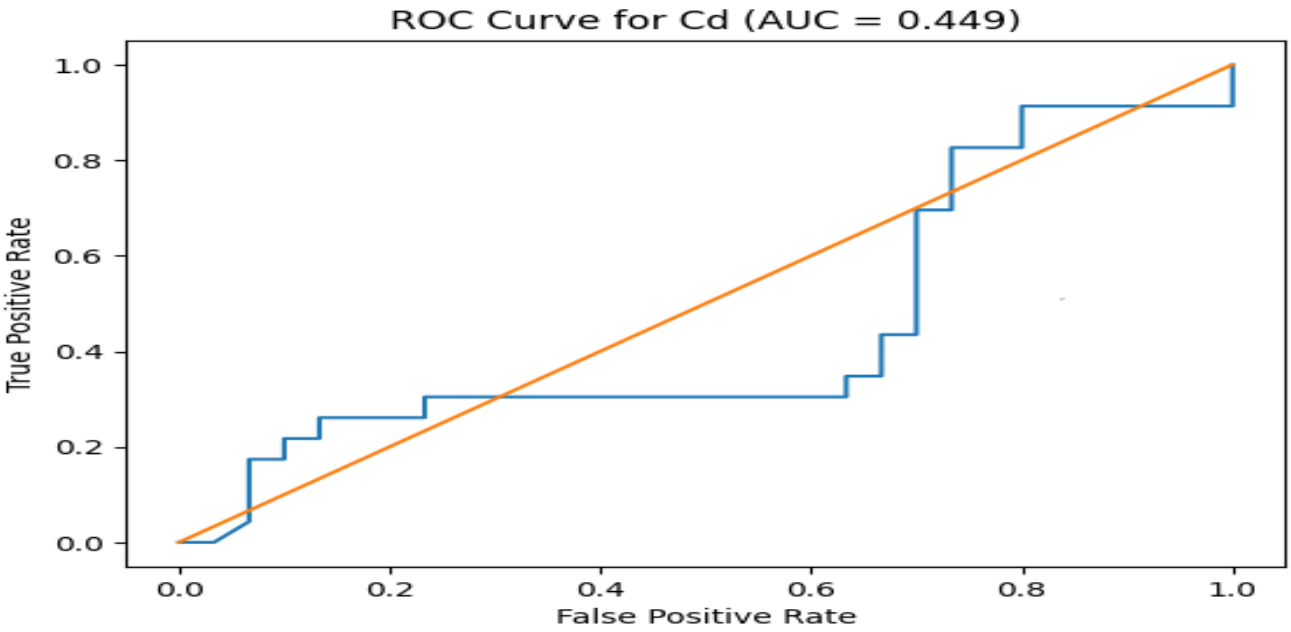

Fig. 7. ROC Curve for Cadmium Concentration in Females.

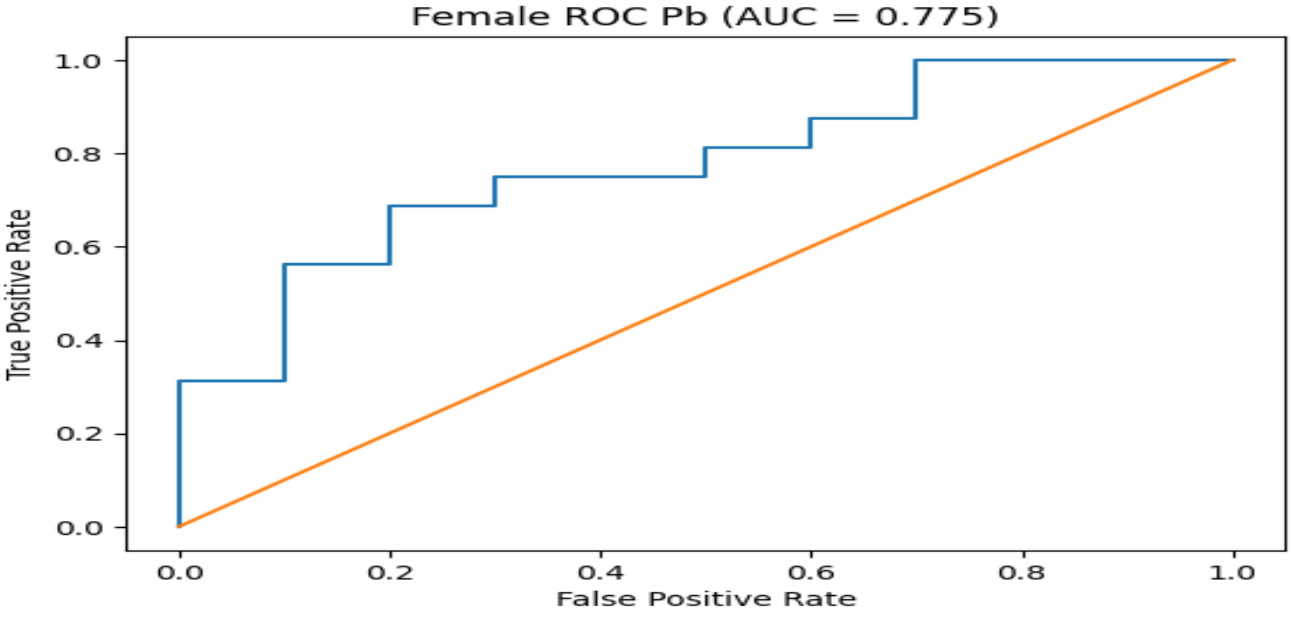

Fig. 8. ROC Curve for lead Concentration in Females.

These findings support the hypothesis that Pb may serve as a useful biological indicator of colon cancer, whereas Cd does not demonstrate sufficient diagnostic reliability.

Table 4. ROC Analysis of Cadmium (Cd) and Lead (Pb) for patients in males.

Element	AUC	Discriminatory Power	Cut-off
Cd	0.449	Very poor	0.437
Pb	0.784	Good	2.248

Table 5. ROC Analysis for Cadmium (Cd) and Lead (Pb) for patients in Females.

Variable (Female)	AUC	Discriminatory Power	Cut-off
Cd	0.344	Very poor	0.038
Pb	0.775	Good	1.843

4.5 Two-Way ANOVA Analysis

A two-way analysis of variance (ANOVA) was performed to examine the combined effects of gender and colon cancer Pb and Cd concentrations. Exposure significantly affects lead concentrations, as demonstrated in the analysis of lead levels in colon cancer ($p < 0.001$)[22]. However, no statistically significant interaction was found between gender and other factors. Consequently, elevated lead levels in colon cancer patients consistently occur in both males and females. Regarding cadmium levels, no statistically significant effects of gender or other factors were found; therefore, cadmium concentrations are not strongly associated with colon cancer in this study.

Table 6. Comparison of Statistical Significance and ROC Values for Pb and Cd According to gender.

Variable	Males	Females
Cd	Non-significant	Non-significant
Pb	Significant	Significant
ROC (Pb)	0.784	0.775

4.6 Logistic Regression Analysis

The results obtained from multivariate logistic regression analysis to estimate lead, cadmium, and gender levels in patients indicated that lead was the strongest independent predictor, with a probability ratio (OR) of approximately 4.12. Cadmium showed no statistically significant association with cancer status, suggesting that it is not a reliable predictor in the current model. Gender had a moderate effect, but less pronounced than lead concentration. The good discriminatory performance of the logistic regression model, with an AUC of 0.808, indicates a high level of classification accuracy.

Table 7. Multivariate Logistic Analysis Depending variable: Workers Independent variables: Pb+ Cd+ gender.

Variable	Odds Ratio (OR)	Interpretation
Pb	4.12	Each unit increase in lead concentration increases the odds of cancer by 4.12 times
Cd	0.74	Not statistically significant; no meaningful effect on cancer
Gender (female vs male)	3.35	Females have a relatively higher odds of patients compared to males

Model AUC	0.808	Excellent model performance; good discrimination between cases and non-cases

4.7 Overall Interpretation

The results indicated a continued increase in lead levels among patients compared to the control group, confirming a strong association between lead (Pb) concentration and colon cancer. This strong correlation between lead levels and colon cancer was supported by multiple statistical analyses, including non-parametric tests, ROC curve analysis, ANOVA, and logistic regression.

While the statistical analyses didn't show any statistically significant differences between the groups with respect to cadmium, meaning that its diagnostic performance was weak, cadmium cannot be relied upon as a biomarker for colon cancer in this study.

5. Conclusion

The significant increase in lead (Pb) concentration in colon cancer patients (both male and female) compared to the control group, as documented in the study results, may indicate a potential influence on disease development or progression. Lead exhibited good discriminatory power between cancer patients (of both genders) and controls (AUC ≈ 0.78), suggesting that the effect of cancer is gender-independent. Cadmium, on the other hand, did not demonstrate this characteristic; in other words, its diagnostic power was very weak (AUC < 0.5). This means that statistically significant differences in cadmium (Cd) concentrations were not well-defined between the patient and control groups, indicating an unclear association between cadmium and cancer in this study.

Two-way ANOVA analysis showed that the most significant factor influencing elevated lead concentrations was cancer, not gender.

Multivariate logistic regression analysis showed that lead is the strongest predictor of cancer. Overall, the results suggest that lead can be considered an important biomarker.

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