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To Study the Circulating Tumor Cells Filtered from The Breast, Colon and Prostate Cancer Patients Blood Using Multi-Color Flow Cytometry

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Abstract

Background: Cancer ranks as a leading cause of death and an important barrier to increasing life expectancy in every country of the world. Tumour cells that have separated from the main tumour and permeated the bloodstream are known as circulating tumour cells (CTCs). These cells are indicators of tumour diagnosis, prognosis, residual disease, and metastasis because they share antigens or genetics with a particular type of tumour. One uncommon subpopulation of cells called CTCs serves as a seed for metastasis. CTCs are promising biomarkers for determining the current tumour status and potential for metastasis is thought to be CTCs. **Objectives:** To study the circulating tumor cells filtered from the breast, colon and prostate cancer patients blood using multi-color flow cytometry. **Material and Methods:** This was a Prospective Study carried out in a Tertiary Care Centre. The study duration was 1 year, and the period was from November 2022 to November 2023 with the department of Pathology after obtaining clearance from the institutional ethics committee and written informed consent from the study participants. A total sample of 20 was used which included 5 cases of breast, colon and prostate cancer each and 5 healthy controls. 15 ml of peripheral blood was collected from 20 study participants using EDTA coated vacutainer under aseptic conditions. The percentage of cells was analysed by using flow cytometry. A p-value of <0.05 was considered statistically significant. **Results:** About three-fifth of study participants were males (60%) and two-fifth (40%) were females. The mean age for the study group was 67 with a standard deviation of 10.71 years. Mean value of CD133 and CD44 was considerably higher among cancer patients as compared to healthy controls. **Conclusion:** The elevated expression of CD 44and CD 133 in different types of cancer patients is a promising marker for early diagnosis and disease prognosis. These findings may provide critical information for developing novel cancer and treatment strategies.

Keywords: Cancer stem cells, Circulating tumor cells, CD44, CD133, Metastasis.

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Introduction

In 21st century, Cancer has emerged as the one of the major public health problem across the globe. It exerts a significant impact on everyone involved ranging from individual affected, to their families, communities and healthcare systems. Apart from these, cancers are one of the leading cause of morbidity and mortality among the general population. Rising trends of cancers could be contributed to many factors such as changing demographics of population, lifestyle changes such as unhealthy diet, physical inactivity, tobacco and alcohol use, environmental pollution and exposure to different carcinogens agents.[1]

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According to GLOBOCON 2020, an estimated 19.3 million cases occurred throughout the world and projections estimate them to rise to 28.4 million cases by 2040, a rise by 47% from 2020 levels.[2] Cancer ranks as a leading cause of death and an important barrier to increasing life expectancy in every country of the world.[3] According to estimates from the World Health Organization (WHO) in 2019, cancer is the first or second leading cause of death before the age of 70 years in 112 of 183 countries and ranks third or fourth in a further 23 countries.[4]
Circulating tumor cells (CTCs) are tumor cells in circulation. Tumor cells that shed from the primary tumor site and intravasate into the peripheral blood circulation are responsible for the formation of secondary tumor or metastasis.[5] They are a rare subset of cells which act as a seed for metastasis.[6] They provide a blood biomarker for early carcinogenesis, cancer progression and treatment effectiveness. They have been considered as one of the promising biomarkers to give information of current tumor status and metastasis potential.[7] They are considered to be precursors of metastasis, as metastasis of cancer cells is the major cause of death, and it is incurable isolation of CTCs can be used as non-invasive biomarkers predicting therapeutic response.[8]
The present study was conducted to study the circulating tumor cells (CTCs) filtered from the breast, colon and prostate cancer patients blood using multi-color flow cytometry and the comparison of expression of CD44 and CD133 over these cells against healthy controls.

Methodology

Patient Selection

This was a prospective study carried out in a Tertiary Care Centre situated in western part of India. The study duration was 1 year, and the period was from November 2022 to November 2023 with the department of Pathology. A total sample of 20 was used which included 5 cases of breast, colon and prostate cancer each and 5 healthy controls. Inclusion criteria to be part of the study was that patient must be aged between 18 to 80 years and giving written informed consent. Only biopsy proven cancer patients were taken as study participants. Prior to start of study, permission from institutional ethics committee (IEC) was taken.

Blood Samples

15 ml of peripheral blood was collected from 20 study participants using EDTA coated vacutainer under aseptic conditions.

Processing of Samples

Whole blood collected in EDTA coated vacutainer underwent processing and the mononuclear cells were separated through Ficoll – density gradient method and centrifugation. Diluted whole blood is layered on top of a density gradient medium and is centrifuged to separate distinct cell populations, causing erythrocytes and granulocytes to settle at the bottom of the tube and mononuclear cells including CTCs to remain above the gradient which remains accessible for collection and analysis. (Figure 1)

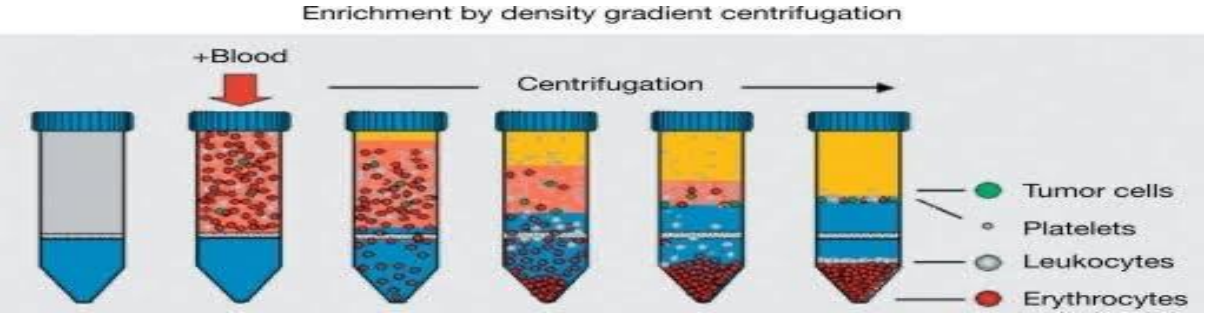

Figure 1: Processing of Samples

Statistical Analysis

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Collected data was entered into MS-Excel and was analyzed using Statistical package for Social Sciences (SPSS) version 25.0. Categorical data was expressed as frequency or proportions and quantitative data as mean and standard deviation. Chi-square test or Fisher's exact test were used as test of significance for categorical variables and unpaired t-test was used for mean and standard deviation. A p-value of <0.05 was considered statistically significant.

RESULTS

The expression of the cancer stem cell (CSC) markers CD133 and CD44 was examined in the samples received from study participants which comprised of a total of 20 individuals. Out of these 20 individuals, 5 were healthy controls and 15 cancer patients (breast cancer, n=5, colon cancer, n=5 and prostate cancer, n=5). Table 1 shows the sociodemographic characteristics for our study. About three-fifth of study participants were males (60%) and two-fifth (40%) were females. The mean age for the study group was 65 with a standard deviation of 9.07 years. The majority of study participants were in the age group of more than 60 years (70%).

Table 2 shows the comparison of mean values of CD133 and CD44 among healthy controls and cancer patients. It can be observed from the table that mean value of CD133 and CD44 was considerably higher among cancer patients as compared to healthy controls. Being a quantitative (continuous) variable, the normality of distribution was tested using Shapiro-Wilk test and it was found to have non-parametric distribution and therefore, Mann-Whitney U test was used as statistical test of significance. On applying Mann-Whitney U test, the difference between mean values for CD133 among healthy controls and cancer patients was found to be statistically significant ($p = 0.001$). Similarly, for CD44, the difference was also statistically significant ($p = 0.002$).

On further sub-group analysis for comparison among healthy controls and different types of cancer patients, all cancer types which were part of the study had significantly higher levels of CD133 and CD44 expression ($p < 0.05$). (Table 3)

Table 1: Sociodemographic Characteristics

Variables	Frequency (N = 20)	Percentage (%)
Gender		
Males	12	60
Females	08	40
Age		
<60 years	06	30
> 60 years	14	70
Age (Mean ± SD) (years)	65 ± 9.07	

** SD – Standard deviation*

Table 2: Comparison of mean values of CD133 and CD44 among healthy controls and cancer patients

Antibody	Healthy Controls	Cancer Patients	U statistic, p value
CD133 (Mean ± SD)	0.03 ± 0.03	0.41 ± 0.26	0.00, 0.001
CD44 (Mean ± SD)	0.03 ± 0.02	0.35 ± 0.32	1.00, 0.002

** p value < 0.05 statistically significant; Mann Whitney U test applied*

Table 3: Sub-group Analysis for expression of cell surface markers

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Type of Cancer	CD133 (Mean ± SD)	CD44 (Mean ± SD)
Breast Cancer	0.36 ± 0.13	0.31 ± 0.11
Prostate Cancer	0.22 ± 0.11	0.22 ± 0.08
Colon Cancer	0.65 ± 0.25	0.52 ± 0.46

DISCUSSION

One of the most significant advances in cancer biology in recent decades has been the identification of cancer stem cells, or CSCs. Tumor-initiating cells, or CSCs, are a small subset of cancer cells that are thought to be in charge of the development, metastasis, and start of tumours. They are also to blame for other patients' cancer recurrences. In this study, we examined the expression of two well-known CSC markers in healthy controls and several cancer patient types: CD44 and CD133.

We found that there was a positive association between the levels and the severity of the disease, and that the levels were significantly greater in cancer patients than in healthy controls. These results highlight the role that CSCs and their markers play in the initiation and spread of cancer, and they may also provide insight into the potential utility of CSC markers for therapeutic categorization and early diagnosis. This could also provide insight into the patients' prognosis and treatment plans. The findings of this extensive prospective research by Rack B et al involving patients diagnosed with primary breast cancer indicate the independent prognostic significance of CTCs before to and following adjuvant chemotherapy.[9]

In another study by Trapp E et al, two years following treatment, the presence of CTCs was linked to a lower overall survival (OS) and Disease Free Survival (DFS). These findings call for a reevaluation of biomarker-based active, tailored surveillance programmes for breast cancer survivors.[10]

Cieślikowski WA et al. conducted a study whose findings imply that high CTC counts may help identify high-risk prostate cancer patients with occult metastases at the time of diagnosis, even though the sensitivity of CTC detection needs to be further improved.[11]

The identification of CSCs has raised the possibility of creating novel cancer therapies that specifically target CSCs markers. We could eliminate the source of cancer cells, avoiding relapse and disease development, by locating and killing these cells

There is no study without any limitations, and this also has few. One of the limitations of the present study is that the sample size analyzed was too small and therefore, results of study could not be generalized. Secondly, since the study includes only patients presenting to the hospital, it may lead to missing out many similar cases which being not reported at the time of the study. Thirdly, the study only looked at two particular markers, and more research is required to find and validate more CSC indicators that could offer deeper insights into the biology of CSCs.

CONCLUSION

This study demonstrates that cancer patients expressed CD44 and CD133 at significantly higher levels than healthy controls. across addition to providing new insight into the biology of the illness, the observed elevation of cancer stem cell markers across a variety of cancer types may have important implications for the development of novel therapeutic and diagnostic strategies.

Different types of cancer patients have higher expression of CD44 and CD133, which is a promising marker for early diagnosis and prognosis. These results might offer vital information for creating cutting-edge methods of cancer detection and treatment. Research on CTCs has the potential to shed light on the basic mechanisms underlying metastases, such as how CTCs extravasate from the original tumour, interact with blood cells to survive in the circulating microenvironment, and intravasate into the distant metastatic site to form new lesions.

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 12, 2023

Important molecular characteristics of CTCs can help pinpoint treatment targets for metastatic cancer. Since only a tiny percentage of CTCs can eventually develop into metastases, research concentrating on these highly metastatic CTCs may offer more profound understanding of therapeutic targets connected to CTCs.

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