Utility of blood DNA levels in diagnosis of breast cancer

ABSTRACT

Background: Prognostic factors, including both histopathological and biochemical variables, influence the choice of modality and the course of therapy in breast cancer. The biomarkers found in biological fluids, particularly in blood, apparently hold the best promise for the development of screening assays.

Aim: To find out if any correlation exists between blood DNA level and tumor stage, size and grade.

Materials and Methods: This case-control study was carried out on 52 female patients in the age-group of 18-70 years. The cases comprised 25 patients with histopathologically confirmed malignant breast cancer, while 27 patients with benign breast tumors served as the control group.

Statistical Analysis: We used the Student’s ‘t’ test to compare the differences between the blood DNA levels in the two groups. Pearson’s test was performed to find out the correlation between blood DNA levels and the TNM stage, tumor size and grade.

Results: It was observed that blood DNA levels showed statistically significant correlation with the TNM stage, tumor size and grade.

Conclusion: The blood DNA level can be utilized as a noninvasive marker to assess tumor aggressiveness. Thus, it can be useful as a prognostic marker and as a marker of tumor burden.

KEY WORDS: Breast cancer, DNA, tumor
cases and controls. The DNA was extracted by salt-chloroform method. The EDTA blood sample was mixed with lysis buffer (proteinase K, sodium chloride, EDTA, and sodium dodecyl sulphate) and incubated at 55°C-65°C with periodic mixing. After incubation, saturated NaCl and chloroform were added; this was mixed thoroughly for 10 min by inverting the tube repeatedly and the mixture was then centrifuged. The upper aqueous phase was used for precipitation of DNA. The DNA was precipitated by ethanol and estimated colorimetrically, using the diphenylamine method. All the reagents used in the estimation were of analytical grade.

Statistical analysis
We used Student’s ‘t’ test to assess the significance of the difference between the mean blood DNA levels in benign and malignant cases. Pearson’s correlation was used to study the relationship between the variables in the study.

RESULTS
Student’s ‘t’ test was performed to determine the significance of the difference between the mean levels of the variable in controls (benign) and cases (malignant). A cut-off value of 40 mg/l was used for statistical tests.

From Table 1 it can be seen that there is a significant difference between the mean blood DNA levels in the benign and malignant groups (t = 7.17; P = 0.000). In addition, blood DNA levels were elevated to twice the cut-off value in 36% of malignant tumors.

In order to determine how the level of the marker changed with progressive clinical disease, we performed a correlation test of blood DNA level against TNM staging. There was a positive correlation of 0.81 between the level of the marker and the TNM stage, suggesting that the marker closely follows the clinical picture.

Pearson’s test was also performed to ascertain if the blood DNA values correlated with tumor burden. We found a positive correlation between the marker and the tumor size (r = 0.69) [Figure 2]. A positive correlation was also seen between blood DNA values and tumor grade, with a correlation value of 0.51 [Figure 3].

DISCUSSION
Nucleic acid level in blood, particularly that of DNA, has assumed importance as a marker of malignancy. It has been found to be useful in the diagnosis, prognostication, and monitoring of cancer.

In our study, the mean blood DNA level was significantly higher in the group with malignant tumors as compared to the control group, with a t value of 7.17 and P = 0.000. This is similar to the findings obtained by Leon and others, who reported a higher concentration of serum DNA in cancer.

Table 1: DNA level in malignant and benign tumors

<table>
<thead>
<tr>
<th>DNA level</th>
<th>Malignant tumors</th>
<th>Benign tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases</td>
<td>25</td>
<td>27</td>
</tr>
<tr>
<td>Minimum (in mg/l)</td>
<td>24.00</td>
<td>8.0</td>
</tr>
<tr>
<td>Maximum (in mg/l)</td>
<td>166.40</td>
<td>28.80</td>
</tr>
<tr>
<td>Mean (in mg/l)</td>
<td>78.42</td>
<td>18.54</td>
</tr>
<tr>
<td>SD</td>
<td>43.00</td>
<td>5.63</td>
</tr>
<tr>
<td>Student’s ‘t’ value</td>
<td>t = 7.17</td>
<td></td>
</tr>
<tr>
<td>Probability</td>
<td>P = 0.000</td>
<td></td>
</tr>
</tbody>
</table>

![Figure 1: Relationship between DNA and tumor stage](image1)

![Figure 2: Relationship between DNA and tumor size](image2)

![Figure 3: Relationship between DNA and tumor grade](image3)
patients when compared to normal individuals. With the cut-off at 40 mg/l, it is found that around 36% of patients with malignancy showed DNA levels more than twice the cut-off. The sensitivity and specificity of the marker in breast cancer has been found to be 92.0% and 88.9%, respectively, while the positive and negative predictive values were found to be 86.9% and 93.1%, respectively. This implies that, with a cut-off value of 40 mg/l, 86.9% of patients with elevated blood DNA values are likely to have malignancy. Similarly, 93.1% of patients with normal blood DNA levels are likely not to have malignancy. In summary, since blood DNA levels are found to be elevated in patients with malignant tumors, it has the potential to be a sensitive and specific marker in malignancy of the breast. Among patients with malignant tumors, the mean DNA level increased with the TNM stage, with a correlation coefficient of 0.81. As the TNM stage increases, the prognosis becomes poorer and, therefore, the blood DNA level could be used as a prognostic marker. As the size of the tumor increases, the blood DNA level also increases and thus serves as a marker of the tumor burden. There is also a positive correlation (r = 0.51) between the blood DNA level and the grade of differentiation of the tumor. Increase in tumor grade implies poor differentiation of the cells in the tumor.[11] Higher blood DNA levels are found in cases of higher grade, i.e., tumors which are ill-differentiated. Such tumors carry a poor prognosis. Thus, DNA levels could also be used to predict the prognostic outcome in patients. The findings of this study are consistent with other studies on plasma DNA at the molecular level in cases of pancreatic carcinoma,[12] non-small-cell lung cancer,[13] melanoma,[14] and hepatocellular carcinoma.[15]

In the present study, DNA levels in plasma are significantly higher in patients with malignant breast tumor than in those with benign breast tumor with good analytical performance. DNA levels in blood correlate with histopathological variables like stage, size, and grade of the tumor in breast cancer, indicating that it has the potential to be a useful marker of tumor burden. The DNA concentration in blood obtained preoperatively could serve as a relatively noninvasive marker to assess tumor aggressiveness. Thus, the blood DNA level, which reflects the clinical status, could help the clinician to adopt the appropriate therapeutic measures.

REFERENCES