Exploring the Relationship Between CD 166 Expression and BI-RADS scores in Breast cancer patients and Healthy Volunteers

Vemareddy Hemalatha 1, Bhawna Dev 2, Nagasubramanian Vanitha Rani 3, Rajendran SD 4, Shabna Roupal 5

1. Faculty of Pharmacy, Sri Ramachandra Institute of Higher Education and Research, Chennai, IND 2. Department of Radiology, Sri Ramachandra Institute of Higher Education and Research, TamilNadu, IND 3. Pharmacy Practice, Jaya College of arts and science College of Pharmacy, Chennai, IND 4. Pharmacy, Scitus pharmaceuticals, TamilNadu, IND 5. Faculty of Pharmacy, Sri Ramachandra Institute of Higher Education and Research, TamilNadu, IND

Corresponding author: Shabna Roupal, shabnaroupalmorais@gmail.com

Abstract

Background: This research embarked on a crucial endeavor to clarify the connection between levels of CD166 expression and the established BI-RADS grading system. Through a comprehensive exploration of this correlation, the objective was to ascertain if CD166 could function as an additional biomarker, enhancing the predictive effectiveness of the BI-RADS classification.

Method: This prospective observational study involved 81 women with histopathologically confirmed early breast tumors and 81 radiologically confirmed healthy breast volunteers. The BI-RADS scores of all the participants included in the study were recorded. Before starting treatment, serum, saliva, and urine samples were collected. The CD166 levels were quantified using an enzyme-linked immunosorbent assay.

Results: The study involved the analysis and comparison of the mean and standard deviations of CD166 expression in serum, saliva, and urine across various BI-RADS Categories. Notably, statistically significant differentiation was found (p=0.00) across all samples spanning the spectrum of BI-RADS categories.

Conclusion: A progressive rise in CD166 concentration coincides with the increasing gradient of the BI-RADS Category, implying a possible link between CD166 and breast cancer progression and severity.

Introduction

Introduction:

Breast cancer (12.5%) remains a formidable global health issue, affecting millions of women globally. For effective treatment planning and patient outcomes, early detection and precise risk assessment are critical. [1] According to the latest statistics on breast cancer in India, the estimated number of new cases in 2018 in India, is 1,62,468 (27.7%), and mortality is 87,090 (23.5%). [2] The data obtained from different breast cancer registries across the country reflects the incidence varying from 30.7% in Chennai to 19% in Dibrugarh. [3]

The Breast Imaging Reporting and Data System (BI-RADS) (5th Edition BI-RADS Atlas) is a standardized classification system for mammography findings and determining breast cancer risk. [4-5] The detailed categories of BI-RADS are mentioned in Table 1. [6] While BI-RADS is useful, there is a rising interest in investigating new biomarkers that could supplement established diagnostic techniques and improve prognosis accuracy.

Cluster Differentiation (CD166), also known as active leukocyte cell adhesion molecule (ALCAM), is a transmembrane glycoprotein that is involved in a variety of biological functions such as cell adhesion, migration, and signaling. [7] CD166 appears to be implicated in cancer progression and metastasis, making it a possible candidate for detecting high-risk breast cancer patients. [8-9]

The purpose of this study is to look into the relationship between CD166 expression levels and the BI-RADS grading system in breast cancer patients. By investigating the relationship between CD166 and BI-RADS, we hope to find out whether CD166 can be used as a valuable adjunct biomarker to improve the predictive capacity of the BI-RADS classification, ultimately assisting in early diagnosis, risk stratification, and personalized treatment approaches for breast cancer patients.

Our work intends to shed light on the possible significance of CD166 in breast cancer diagnosis furthering
our understanding of the disease and potentially paving the way for improved patient care and outcomes.

**Materials And Methods**

Methodology: This prospective observational study was conducted at Sri Ramachandra Medical Centre, Chennai, with approval from the institutional ethics committee of Sri Ramachandra Institute of Higher Education and Institute, Chennai (IEC No: IEC-NI/20/FEB/74/24).

Sample Size: With expected sensitivity of 70%, with a precision of 10 & at a 95% Confidence interval, the minimum sample size required was 81 female subjects confirmed with breast cancer (case group) and 81 ages matched healthy volunteers as the control group.

Case Group: A total number of 81 women with histopathologically proven malignant early breast tumors up to 3 cm in size were included.

Control group: Healthy women volunteers with no evidence of any health issues or breast abnormalities, as confirmed by mammography or breast ultrasound, were included in the control group.

To assure the baseline assessment of the study participants (Case group), all samples (Serum, Saliva, and Urine) were obtained before the commencement of any treatment, such as neo-adjuvant chemotherapy or breast conservation surgery (BCS).

From all the included study participants from both the case group and control group, serum, saliva, and urine samples were collected, cool centrifuged and the supernatant was stored at -80 degrees C.

From each study participant, relevant clinical data, including age, menopausal status, and any relevant medical history, were gathered for each participant. All patients included in study with breast cancer had histopathological examinations, and their BI-RADS scores were calculated based on mammography findings. All the control patients also had a BI-RADS scoring and BI-RADS values vary from 0 to 6, with higher scores indicating a higher likelihood of cancer. These scores were recorded by qualified radiologists who followed the Atlas 5th edition BI-RADS recommendations.

**CD166/ALCAM expression analysis:**

The collected biological samples from breast cancer patients and healthy female volunteers were analyzed for ALCAM concentration by using Human ALCAM ELISA kit (Abbkine, Korea). By using the Enzyme-linked immunosorbent assay method, CD166 levels were quantified.

**Statistical analysis:**

The collected data, including BI-RADS scores and CD166 expression levels, were entered into a statistical software package for analysis. Descriptive statistics, such as means, standard deviations, and frequencies, were calculated for demographic variables and BI-RADS scores. One-way ANOVA was used to assess the CD166 expression in different BI-RADS categories (1-5).

**Results**

A total number of 162 patients were included in the study, 81 patients were radiologically as well as histopathologically confirmed breast cancer patients and 81 patients were radiologically confirmed with healthy breast. All the study participants were given different BI-RADS scores based on the ACR Atlas 5th edition BI-RADS category by experienced radiologists.

When we conducted a comparative analysis between various BI-RADS categories (1-5) and the corresponding serum CD166 expression for each category, a statistically significant distinction was observed (p=0.00) as depicted in Table 2. Similarly, a notable statistical difference (p=0.00) was evident in Table 3 and Table 4, when we juxtaposed diverse BI-RADS categories (1-5) against the CD166 expression levels in saliva and urine respectively.

Figure 1 graphically elucidates the progressive augmentation of CD166 concentration congruent with the ascending gradient of the BI-RADS category. This visual representation further reinforces the observed association between BI-RADS categorization and CD166 expression levels.
FIGURE 1: A line chart explains CD166 expression in different BI-RADS category patients

CD166: Cluster differentiation 166, ALCAM: Activated leukocyte cell adhesion molecule, BI-RADS: The Breast Imaging Reporting and Data System, pg/ml: Pico gram per ml

<table>
<thead>
<tr>
<th>BI-RADS Category</th>
<th>Impression</th>
<th>BI-RADS Category</th>
<th>Impression</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Mammography: Incomplete – Need Additional Imaging Evaluation and/or Prior Mammograms for Comparison Ultrasound &amp; MRI: Incomplete – Need Additional Imaging Evaluation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Benign (0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Probably Benign (&lt;2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Suspicious Mammography &amp; Ultrasound</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4A Low suspicion of malignancy (2-10%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4B Moderate suspicion of malignancy (10-50%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4C High suspicion of malignancy (51-95%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>High suggestive of malignancy (&gt;95%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Known biopsy-proven malignancy</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TABLE 1: ACR BI-RADS Atlas 5th Edition

ACR: American college of Radiology, BI-RADS: The Breast Imaging Reporting and Data System
### TABLE 2: Serum CD166 expression in different BI-RADS categories of included participants

<table>
<thead>
<tr>
<th>Sample</th>
<th>Group</th>
<th>BI-RADS category</th>
<th>N=162</th>
<th>Mean CD166 expression (pg/ml)</th>
<th>SD</th>
<th>F</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>Control Group</td>
<td>1</td>
<td>45</td>
<td>36.9</td>
<td>11.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>35</td>
<td>36.5</td>
<td>12.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>1</td>
<td>45.9</td>
<td>11.9</td>
<td>37.456</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Case Group</td>
<td>4</td>
<td>35</td>
<td>79.4</td>
<td>12.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>46</td>
<td>77.6</td>
<td>27.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CD 166: Cluster differentiation 166, BI-RADS: The Breast Imaging Reporting and Data System, N: No. of study Participants, pg/ml: Pico gram per ml, SD: Standard deviation, F value: The ratio of between group variation and within group variation, P value ≤0.05 considered as Statistically significant.

### TABLE 3: Saliva CD166 expression in different BI-RADS categories of included participants

<table>
<thead>
<tr>
<th>Sample</th>
<th>Group</th>
<th>BI-RADS category</th>
<th>N=162</th>
<th>Mean CD166 expression (pg/ml)</th>
<th>SD</th>
<th>F</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saliva</td>
<td>Control Group</td>
<td>1</td>
<td>45</td>
<td>36.8</td>
<td>11.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>35</td>
<td>32.8</td>
<td>10.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>1</td>
<td>28</td>
<td>12.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Case Group</td>
<td>4</td>
<td>35</td>
<td>86.6</td>
<td>19.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>46</td>
<td>82.8</td>
<td>15.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CD 166: Cluster differentiation 166, BI-RADS: The Breast Imaging Reporting and Data System, N: No. of study Participants, pg/ml: Pico gram per ml, SD: Standard deviation, F value: The ratio of between group variation and within group variation, P value ≤0.05 considered as Statistically significant.

### TABLE 4: Urine CD166 expression in different BI-RADS categories of included participants

<table>
<thead>
<tr>
<th>Sample</th>
<th>Group</th>
<th>BI-RADS category</th>
<th>N=162</th>
<th>Mean CD166 expression (pg/ml)</th>
<th>SD</th>
<th>F</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>Control Group</td>
<td>1</td>
<td>45</td>
<td>42.3</td>
<td>11.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>35</td>
<td>44.5</td>
<td>13.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>1</td>
<td>32.0</td>
<td>13.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Case Group</td>
<td>4</td>
<td>35</td>
<td>76.9</td>
<td>22.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>46</td>
<td>84.9</td>
<td>21.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CD 166: Cluster differentiation 166, BI-RADS: The Breast Imaging Reporting and Data System, N: No. of study Participants, pg/ml: Pico gram per ml, SD: Standard deviation, F value: The ratio of between group variation and within group variation, P value ≤0.05 considered as Statistically significant.

### Discussion

To the best of our knowledge and research, this is the first study that included 162 individuals to explore the relationship between BI-RADS categories vs. serum, saliva, and urine CD166 expression, so currently this study is short for an original article. The study’s methodology was meticulously designed, involving breast cancer patients (N=81) and healthy volunteers (N=81). Serum, saliva, and urine samples were collected to comprehensively explore CD166 expression.

Among the participants, 81 had histopathologically confirmed breast cancer, while the remaining 81 were radiologically determined as having healthy breasts. The BI-RADS scores assigned to each participant were determined by expert radiologists using the ACR Atlas 5th edition BI-RADS category.
Previous investigations have demonstrated an upregulation of CD166 in breast cancer cases, highlighting a notable distinction in serum CD166 expression between breast cancer patients and healthy volunteers and concluded CD166 diagnostic accuracy. [10-16]

Our findings revealed a significant and persuasive discrepancy (p=0.00) in the association between BI-RADS categories and serum CD166 expression, as seen by the results shown in Table 2. Furthermore, the research into CD166 expression levels in saliva and urine, stratified by separate BI-RADS groups (1-5), revealed a significant statistical divergence (p=0.00), as shown in Tables 3 and 4. These findings highlight the potential of CD166 expression in serum, saliva, and urine as relevant biomarkers for distinguishing differences across distinct BI-RADS categories.

A gradual increase in CD166 concentration corresponds to the increasing gradient of the BI-RADS Category, implying that CD166 may play a role in reflecting breast cancer development and severity.

The primary challenges in this study revolve around identifying individuals with early-stage breast cancer and acquiring biological samples upon immediate diagnosis without any treatment. While the collection and processing of saliva samples pose some difficulties, it’s worth noting that saliva stands out as the optimal specimen for analysis due to its non-invasive nature and cost-effectiveness for patients.

To date, no prior research has assessed or presented data regarding the relationship between CD166 expression and various BI-RADS categories.

Overall, our findings emphasize the importance of CD166 expression as a possible diagnostic marker in the context of BI-RADS assessment, adding to our expanding awareness of its potential utility in the early diagnosis and monitoring of breast cancer. More research and long-term studies are needed to properly understand the clinical significance of these exciting findings and to establish CD166 as a viable addition to breast cancer detection.

**Conclusions**

This study undertook the essential task of elucidating the relationship between CD166 expression levels and the well-established BI-RADS grading system. By delving into this association, the aim was to determine whether CD166 could serve as an adjunct biomarker augmenting the predictive capacity of BI-RADS. Our study findings not only highlight the potential of CD166 expression as informative biomarkers across distinct BI-RADS categories but also point towards its relevance in reflecting the progression and severity of breast cancer.

Importantly, this study contributes as the first of its kind to explore the intricate relationship between CD166 expression and BI-RADS categorization.

**Additional Information**

**Author Contributions**

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

- **Concept and design:** Shabna Roupal, Vemareddy Hemalatha, Bhawna Dev, Nagasubramanian Vanitha Rani, Rajendran SD
- **Acquisition, analysis, or interpretation of data:** Shabna Roupal, Vemareddy Hemalatha, Bhawna Dev, Nagasubramanian Vanitha Rani, Rajendran SD
- **Drafting of the manuscript:** Shabna Roupal, Vemareddy Hemalatha, Nagasubramanian Vanitha Rani
- **Critical review of the manuscript for important intellectual content:** Shabna Roupal, Vemareddy Hemalatha, Bhawna Dev, Nagasubramanian Vanitha Rani, Rajendran SD
- **Supervision:** Shabna Roupal, Vemareddy Hemalatha, Bhawna Dev, Nagasubramanian Vanitha Rani, Rajendran SD

**Disclosures**

**Human subjects:** Consent was obtained or waived by all participants in this study. Sri Ramachandra Institute of Higher Education and Research issued approval IEC-NL/20/FEB/74/24. The study was approved by the institutional ethical committee (IEC-NL/20/FEB/74/24), SRIHER(DU), and informed was obtained before the sample collection. **Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissue. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all
authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** Vemareddy Hemalatha declare(s) a grant from Sri Ramachandra Institute of Higher Education and Research (DU). Shri N.P.V Ramasamy Udayar Fellowship grant from university. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

**Acknowledgements**

Authors were thankful to the Department of Radiology, Sri Ramachandra Medical Centre, Chennai, Tamil Nadu.

**References**