Immunohistochemical Expression of Nibrin in Epithelial Dysplasia and OSCC: A Cross-Sectional Study

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Authors’ contributions

This work was carried out in collaboration among all authors. Author FA designed the study wrote the protocol and wrote the first draft of the manuscript. Authors MH and SAB managed the analysis of the study and literature search. Authors FB and RA read IHC scoring & IHC photography. Author FB helped in manuscript writing. Author ABZ had performed data entry, statistical analysis and result write up. All authors read and approved the final manuscript.

ABSTRACT

Inherited defect in DNA repair capability is the fundamental problem causing mutations to be passed on to new generation of cells leading to cancer. The NBS1/Nibrin/p95 belongs to the family of the DNA double-strand break repair complex (hMre11 complex) which is a transcript of the mutated NBS gene, located on human chromosome 8q21. The protein complex containing nibrin binds to the edges of the DNA double-stranded break causing defective repair process. Nibrin also activates various signaling cascades such as; phosphatidylinositide 3-kinases PI3-kinase/Akt triggering the oncogenic process.
**Aims:** The purpose of this research was to aid in the diagnosis of oral cancer and dysplastic lesions in Karachi population.

**Study Design:** This cross-sectional study was conducted at a tertiary care hospital. All samples were recruited for the study after obtaining written informed consent from patients and seeking ethical approval from Ethic Review Committee Ziauddin University (Reference Code: 0330618FAOM), Karachi Pakistan.

**Place and Duration of Study:** Ziauddin hospital north Nazimabad Karachi, November 2018 to September 2019.

**Methodology:** Expression of Nibrin (NBS1) was evaluated by Immunohistochemistry on dysplasia and OSCC biopsies by H-Score method. The tumor was graded by broder system. Immunohistochemical analysis for nibrin was performed on the tumor block. Mean, frequency, percentages were calculated for quantitative variables, chi square was used for qualitative variables. A value <0.05 was considered as significant.

**Results:** 92% OSCC cases were positive for Nibrin while 80% of oral dysplasia revealed Nibrin expression. This accounts for significant statistical association of Nibrin with OSCC (0.012*) and dysplasia (0.001*). Unfortunately, apart from moderately histological differentiation (0.012) none of these clinicopathological parameters showed statistical significance.

**Conclusion:** We conclude that a Nibrin protein showed overexpression among 92% of OSCC patients out of total 100 cases and in 80% out of 30 cases of dysplasia so the association of histological grading with expression of Nibrin was significant and expression of Nibrin intensity with histological grading was significant as well. Our study suggests that this expression of Nibrin may be used as a diagnostic marker for potential OSCC patients.

**Keywords:** Nibrin; OSCC; dysplasia; immunohistochemistry.

**1. INTRODUCTION**

Squamous Cell Carcinoma is the most commonly occurring cancer in oral cavity, which originates predominantly from buccal mucosa and tongue [1]. In Pakistan the burden of OSCC accounts for 10% of all cancers affecting both genders. This high prevalence of OSCC in Indo-Pak region is mainly contributed by the use of tobacco products, bad habits and poor public health care system. Despite new treatment modalities, the overall survival rate in patient with OSCC has not been significantly improved [2,3].

The development of OSCC is usually preceded by variable levels of cellular proliferation usually graded from mild to severe dysplasia. Over the last decade, scientific research is primarily focused on investigating biological, diagnostic and prognostic parameters linked to the development and progression of dysplasia to OSCC [4,5].

Inherited defect in DNA repair capability is the fundamental problem causing mutations to be passed on to new generation of cells leading to cancer [6]. The NBS1/Nibrin/p95 belongs to the family of the DNA double-strand break repair complex (hMre11 complex) which is a transcript of the mutated NBS gene, located on human chromosome 8q21 [7-9]. The protein complex containing nibrin binds to the edges of the DNA double stranded break causing a defective repair process [10]. Nibrin also activates various signaling cascades such as; phosphatidylinositide 3-kinases PI3-kinase/Akt triggering the oncogenic process [11-13].

Some recent studies have reported aberrant NBS1expression in proliferating oral tissues suggesting a potential to predict carcinogenic process early on [14,15]. However, the status of NBS1 in subset of oral cancer from our population remains unknown. Based on this information, we aim to assess whether the Nibrin expression would be significantly related to OSCC and premalignant dysplasia in tissue biopsies.

**2. MATERIALS AND METHODS**

A cross-sectional study was carried out at Ziauddin University tertiary care hospital using 100 diagnosed OSCC biopsies specimen and 30 samples exhibiting dysplastic changes. All samples were recruited for study after obtaining written informed consent from patients and seeking ethical approval from Ethic Review Committee Ziauddin University (Reference Code: 0330618FAOM), Karachi Pakistan. The patients were recently underwent biopsy for OSCC confirmation during the years 2018- to 2020. The
H & E slides were reviewed by a consultant histopathologist for the selection of most representative tumor block for Nibrin IHC.

The IHC was performed using a commercially available IHC kit for Nibrin expression (Anti-nibrin PA5-22975, Thermo Scientific) following manufacture protocol. Briefly, 3 μm tissue section were cut on standard microtome from selected OSCC block & deparaffinized using xylene, then dehydrated with graded alcohol solutions. Slides were then rinsed with wash buffer and incubated with blocking agent for 15 minutes each. Primary antibodies were then applied at 1:100 dilution followed by overnight incubation. Slides were then washed again with wash buffer and incubated for 30 minutes with secondary antibody and then rinsed again. DAB (Thermo scientific; 750118) solution was then applied and incubated for 20 minutes. Subsequently, sections were washed thrice for 10 minutes each and counterstained with Haematoxylin and mounted with coverslip for evaluation under light microscope.

All positive cases were scored by employing; H – scoring criteria to determine the nuclear expression of Nibrin and interpreted as: Negative (0), Weak (1+), Moderate (2++); more than 25% in tumor cells while strong Intensity (3++++) reflects strong staining in at least 50% of tumor cells. To authenticate the reaction chronic cholecystitis specimen were used as positive IHC control [14].

3. RESULTS

100 biopsy proven OSCC specimen and 30 exhibiting dysplastic changes were used to analyze nibrin IHC expression out of these samples, 92% OSCC cases were positive for Nibrin, while 80% of oral dysplasia revealed Nibrin expression. This accounts for significant statistical association of Nibrin with OSCC (0.012*) and dysplasia (0.001*). Our clinical data showed an overall male predominance in our research samples constituting 77.7% men compared to 22.3% from women. The mean age for OSCC was observed as; 46.96±12 and 50.47±12.94 for dysplasia. Among other clinical variables, the mean tumor size was calculated 3.58 ±2.28 cm and thickness of tumor was 1.50±1.29 cm. Buccal mucosa was the most frequently involved anatomical location for both types of cases in present study.

While comparing the clinicopathological prognostic indicators, majority of OSCC cases were moderately differentiated (87%) and represent clinical stage 4(36%). Variable degree of nodal metastasis was common in current study accounting for 57% OSCC cases involving regional and distant lymph nodes. Unfortunately, apart from moderately histological differentiation (0.012) none of these clinicopathological parameters showed statistical significance.

4. DISCUSSION

Nibrin is a 754 amino acid containing DNA breakdown repairing protein formed by NBN gene. It is identified as an important tri-meric member of NBS1/hMre11/RAD50 (N/M/R), referred as MRN (DNA) DSB break repair complex [14]. Whenever the DNA has been damaged this trimeric complex identifies DNA breakdown and forms nuclear foci at these DSB sites. It also plays an essential role in regulating physiological and mutational changes in DSBs [16,17]. Nibrin act as an tumor suppressor gene as its expression is raised in adjacent normal tissues of OSCC lesions [18].

Our study revealed that OSCC is common among 49-58 years of age group, similar to a previous study which reported that out of 150 OSCC cases predominant age of patients ranged between 41 to 50 years and were highest among all other age groups (30.6% of all cases) [19].

The mean age group of our sample population was found to be 47.77%±12.66% which was consistent with other studies [19-21].

The present study showed a male predominance with 101(77.7%) males with OSCC among a total 130 study participants which was consistent with the majority of the researches conducted in past years all over the world showing a general male dominance [19,20,22,23]. The reason for male predominance is social freedom of males and easy access to the risk factors i.e. carcinogenic substances like tobacco, betel quid, naswar [24].

In our study out of 130 cases Buccal mucosa was the most frequently involved site in 69(69%) cases and the 2nd most common site was lateral border of tongue 12(12%) while 19(19%) of the cases involved other sites. Garavello W et al. 2007 reported similar findings from studies conducted in North America and Europe, where the most common site was tongue followed by buccal mucosa [25]. Similar study findings were
reported in previous study as buccal mucosa was reported as the most common site in 84 cases (56%) followed by lateral border of tongue in 31 cases (21%). Other sites involved were lips, alveolar mucosa, palate and floor of mouth [19]. Similar findings were reported by other studies reported buccal mucosa was the most common site followed by tongue [20,26].

Our study revealed that of a total 100 participants the tumor was moderately differentiated in 87(87%) of the participants followed by well differentiated in 9(9%) and poorly differentiated in 4(4%) of the participants. Which was consistent with other study reported by Alamgir et al. 2016 regarding histology with most (59%) of the participants having moderately differentiated OSCC cases [19].

Table 1. Distribution of Nibrin expression and intensity in all specimens with statistical estimates

<table>
<thead>
<tr>
<th>Specimens</th>
<th>Negative (0) (n=14)</th>
<th>Weak (1+) (n=21)</th>
<th>Moderate (2++) (n=47)</th>
<th>Strong(3++) (n=48)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>OSCC n=100</td>
<td>8(8%)</td>
<td>14(14%)</td>
<td>37(37%)</td>
<td>41(41%)</td>
<td>0.012*</td>
</tr>
<tr>
<td>Dysplasia n=30</td>
<td>6(20%)</td>
<td>7(23.3%)</td>
<td>10(33.3%)</td>
<td>7(23.3%)</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

*Chi-square test significant statistical association
*Significant at <0.05

Table 2. Comparison of Nibrin with clinicopathological parameters and statistical estimates

<table>
<thead>
<tr>
<th>Clinico-pathological parameters</th>
<th>Frequency (n=100)</th>
<th>p -value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;46</td>
<td>49</td>
<td>0.426</td>
</tr>
<tr>
<td>≥46</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>76</td>
<td>0.945</td>
</tr>
<tr>
<td>Female</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Tumor site</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Buccal mucosa</td>
<td>69</td>
<td>0.534</td>
</tr>
<tr>
<td>Tongue</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Tumor thickness (cm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1.5</td>
<td>47</td>
<td>0.575</td>
</tr>
<tr>
<td>≥1.5</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>Tumor size</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1 &lt; 2cm</td>
<td>13</td>
<td>0.095</td>
</tr>
<tr>
<td>T2 &gt;2cm to 4cm</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>T3&gt; 4cm</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>T4 adjacent structure</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Nodal metastasis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N0</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>N1</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>N2a</td>
<td>4</td>
<td>0.479</td>
</tr>
<tr>
<td>N2b</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>N3</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Tumor grading</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 1</td>
<td>9</td>
<td>0.012*</td>
</tr>
<tr>
<td>Grade 2</td>
<td>87</td>
<td></td>
</tr>
<tr>
<td>Grade 3</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

*Significant at <0.05; χ2 test was used
Regarding TNM / Staging, our study results found that 36% belong to T4 whereas 20% were T3 tumor size. In nodal involvement, 43% were N0 whereas 16.2% were in N1 status. Moreover 36% of the participants had Stage IV tumors followed by Stage II among 31(31%), Stage III among 20(20%) and Stage I among 13(13%). Our study findings were similar to previous studies by Dave et al. in 2016 who revealed that among 100 oral squamous cell carcinoma patients 40% of patients had Stage IV followed by 22% with Stage II, 20% with Stage I disease and 18% of patients with Stage III disease [14]. Moreover, another study by Zafar M. et al. in 2015 also reported similar findings to present study with 55.7% Stage III/IV tumors followed by 44.2% Stage I/II out of 140 OSCC cases from Karachi [26].

In our study Nibrin intensity among dysplasia cases were found moderate among 10(33.3%), followed by strong and weak among 7(23.3%) each and negative among 6(20%) out of 24 study participants. Nibrin intensity in OSCC patients was found as strong among 48(36.9%), followed by moderate among 47(36.2%), weak among 21(16.2%) and negative among 14(10.8%) out of 130 study participants (100 OSCC 30dysplasia). Analysis of Nibrin showed expression as positive among 116(89%) and negative among 14(10.8%) out of 130 study participants. The association of histological grading with expression of Nibrin was found significant, whereas expression of Nibrin with histological grading was also found significant. Similar findings were reported in a previous study by Hsu et al. 2010 showed that increased in Nibrin expression was significantly associated with increased in tumor size and oral cancer metastasis [15]. Moreover, an earlier study by Ehlers et al. in 2005 also showed similar results as Nibrin was associated with strong tumor severity [27]. Yang et al. reported that overexpression of NBS1 is an independent marker of poor prognosis in poorly differentiated OSCC patients by activating the oncogenic pathway. Kuo et al. reported the prognostic significance of Nibrin in oesophageal squamous cell carcinoma and found that over expression of Nibrin was observed in 28% cases and hence concluded that it can be used as a prognostic biomarker for survival [28]. Nibrin protein plays an important central role in double-stranded breakage repair pathway; oncoproteins are responsible for the activation of mutated nibrin which further induces the epithelial mesenchymal transition increasing the invasiveness and metastasis in the later stages of head and neck cancer [29]. Another study by Jigna H Dave et al. in 2016 also showed similar findings as significant inverse-correlation of Nibrin over expression was associated with tumor size and tumor stage [14].

Fig. 1. Immunohistochemical expression of Nibrin in OSCC and Dysplasia
Photomicrographs (B,D,F,H) showing H & E staining of OSCC Tissue & dysplasia section slides; 10x Magnification and NBS1 Immunostaining (A,C,G,E) sections showing cytoplasmic and membrane immune reactivity (A) Weak, (C) Moderate and (E) Strong immune reactivity). Section G showed moderate dysplasia
5. CONCLUSION

We conclude that a Nibrin protein showed overexpression among 92% of OSCC patients out of total 100 cases and in 80% out of 30 cases of dysplasia so the association of histological grading with an expression of Nibrin was significant and expression of Nibrin intensity with histological grading was significant as well. The study suggests that this expression of Nibrin may be used as a diagnostic marker for potential OSCC patients.

CONSENT

Written consent was obtained.

ETHICAL APPROVAL

Ethical approval was obtained from Ziauddin University Ethics Review Committee (Reference Code: 0330618FAOM).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/66873