INTRODUCTION

Bladder cancer (BCa) is now recognized as the 7th most common malignancy in men worldwide and is most commonly diagnosed in developed countries [1,2]. Even though predominantly found in developed countries, mortality rates are greater in developing countries [3]. The higher mortality rate is thought to be due to less availability of high-quality facilities in under-developed countries to properly diagnose and follow-up BCa. As a result, the time of diagnosis of BCa is considerably later with a predominance of the higher stage (more than T2), which is more common in less developed countries [4].

The current treatment options for patients with bladder cancer are differentiated according to the invasion of cancer cells into the muscle layers of the bladder; the universal strategy of one size fits all is now considered obsolete regarding most other cancers [5]. Radical cystectomy and extended lymphadenectomy for MIBC can significantly improve the 5-year survival rates and are defined as the standard of therapy for MIBC [5,6]. However, these procedures are associated with high morbidity and complications [7,8]. Thus, a bladder sparing strategy with tri-modal treatment has recently gained popularity [9,10]. This strategy includes bladder preservation with maximal resection of tumor (TUR), perioperative chemotherapy, and radiotherapy. Cisplatin-based is recently the first choice for neo-adjuvant chemotherapy (NAC) for bladder cancer [5], but the oncological outcome still needs improvement. An attempt in improving cisplatin with the addition of bevacizumab was reported to fail in making any improvement [11]. Checkpoint inhibitors are reported to have good durability and tolerability in invasive BCa, and five agents are approved as therapy after cisplatin-based by the FDA. Several studies have shown these agents achieve complete responses [12–16].

Over-expression of Osteopontin as Potential Predictive Biomarker for Bladder Cancer Treatment

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ABSTRACT

Background: Current options for management of bladder cancer (BCa) still depend on traditional clinical determinants of stage and histological grade which often do not reflect the biological entity of the tumors. Therefore, new biomarkers are required to better determine suitable treatments for different types of bladder cancers. Recent research has shown osteopontin (OPN) expressions correlate with clinic-pathological variables and outcomes of bladder cancer. This study aimed to evaluate the expression of OPN in the Indonesian population, and it’s potential role as an immune-targeting therapy of BCa.

Methods: Total RNAs from formalin-fixed paraffin-embedded tissues were extracted from 49 patients with bladder cancer consisting of normal histopathology (n = 4), chronic cystitis (n = 15), non-muscle-invasive bladder cancer (NMIBC, n = 15), and muscle-invasive breast cancer (MIBC, n = 15). The expression of OPN was measured using reverse transcription-polymerase chain reaction.

Results: The baseline clinical and histo-pathological characteristics were not statistically different. The expression of OPN was statistically higher in bladder cancer compared to normal histology tissues (P < .001). The expression of OPN was statistically higher in MIBC compared to NMIBC (P < .001).

Conclusions: The expression of OPN was significantly higher in bladder cancer and compared to NMIBC, the OPN expression in MIBC was significantly higher rendering the potential role of OPN expression as a surrogate biomarker marker to determine suitable treatment options for patients with bladder cancer.
the studies reflect only short-time data. The longest study was conducted within 2 years and involved practices with a high incidence of toxicities. These treatments are typically given until complete response or sign of toxicities [17] and no decisive clinical applicability can be made at this time.

Osteopontin (OPN) is an emerging biomarker for a therapeutic strategy. OPN regulates angiogenesis, cell proliferation, invasion, and metastasis by multiple pathways. Accordingly, targeting this protein is considered to be a promising strategy in treating several solid cancers [18–23]. Some strategies have been introduced to suppress the expression of OPN [22–24] resulting in increased autophagy of cancer cells when giving chemotherapy. In addition, one study showed that metformin treatment was able to diminish the expression of OPN in mouse adipose-derived multipotent stromal cells [25]. Our current study is aimed at evaluating the expression of OPN in primary bladder cancer tissues and exploring its potential role to determine the focus of future treatment of bladder cancer and understand its potential role as a surrogate biomarker in the immune-targeting therapy.

METHODS

Sample collection and exosome isolation

The samples were collected from BCa patients who underwent TURBT and biopsies consisting of 15 MIBCs, 15 NMIBCs, and chronic cystitis. Normal samples (n = 4) from biopsies that were confirmed with histopathological evaluations were included in the analysis as a control. The normal samples were obtained from patients with suspected bladder tumors during periods of studies, and the biopsies revealed normal tissues (well-differentiated urothelial). Chronic cystitis on this study defined as microscopic findings revealed edema and nonspecific inflammatory in the lamina propria. The definitions of MIBC and NMIBC are based on world consensus diagnosed by pathological staging (AUA and EAU). NMIBC is defined as confined tumor to bladder mucosa and submucosa with no involvement of muscle, which is indicated by the pathological staging of tumor after TURBT or en-block incision is less than T2A. Meanwhile, any involvement of muscle is considered as MIBC which indicates T2A tumor or worse.

This study received approval from the Universitas Gadjah Mada Ethical Review Board. Total RNA was isolated from tissue samples using the RNeasy Mini Kit Qiagen (Catalog no. 74104). A total of 0.5 μg RNA was used for the synthesis of cDNA using Qiagen Long Range 2 Step RT-PCR Kit (Catalog no. 205920). 5 μL of total cDNA was mixed with 12.5 μL of 2x SYBR Green PCR mix with ROX from BioRad and 10 pmol/μL of each forward and reverse primer for the measured genes.

OPN gene expressions were quantified using reverse transcription-polymerase chain reaction. For the relative quantification, the target gene expression was determined by the (2−ΔΔCt) method after normalization of the gene of GAPDH for CT values. RNA Isolation and cDNA Synthesis. Clinical data were collected from medical records to evaluate the clinical features and confirmation of the diagnosis.

Statistical analysis

The SPSS 23.0 statistical software (IBM Corporation, Armonk, NY, USA) was used to analyze the data, which were presented as mean ± standard error of the mean (SEM). The homogeneity and normality of the data were tested with Levene and Shapiro-Wilk tests. The statistical significance between groups was calculated using an independent sample T-test.

RESULTS

Table 1. Demographic characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean (±SD)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ages</td>
<td></td>
<td>0.74*</td>
</tr>
<tr>
<td>Normal</td>
<td>63.25 (±6.39)</td>
<td></td>
</tr>
<tr>
<td>NMIBC</td>
<td>58.6 (±12.17)</td>
<td></td>
</tr>
<tr>
<td>MIBC</td>
<td>60.2 (±9.93)</td>
<td></td>
</tr>
<tr>
<td>Cystitis</td>
<td>50.9 (12.6)</td>
<td></td>
</tr>
<tr>
<td>Gender (Male)</td>
<td></td>
<td>0.50**</td>
</tr>
<tr>
<td>Normal</td>
<td>2 (50%)</td>
<td></td>
</tr>
<tr>
<td>NMIBC</td>
<td>10 (66.7%)</td>
<td></td>
</tr>
<tr>
<td>MIBC</td>
<td>9 (60%)</td>
<td></td>
</tr>
<tr>
<td>Cystitis</td>
<td>6 (40%)</td>
<td></td>
</tr>
<tr>
<td>Staging</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MIBC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; T2A</td>
<td>2 (13.3%)</td>
<td></td>
</tr>
<tr>
<td>&gt; T2B</td>
<td>13 (86.7%)</td>
<td></td>
</tr>
<tr>
<td>N0</td>
<td>11 (73%)</td>
<td></td>
</tr>
<tr>
<td>N1</td>
<td>3 (20%)</td>
<td></td>
</tr>
<tr>
<td>N2</td>
<td>1 (6.7%)</td>
<td></td>
</tr>
<tr>
<td>M0</td>
<td>11 (73.3%)</td>
<td></td>
</tr>
<tr>
<td>M1</td>
<td>4 (26.7%)</td>
<td></td>
</tr>
<tr>
<td>Low Grade</td>
<td>3 (20%)</td>
<td></td>
</tr>
<tr>
<td>High Grade</td>
<td>12 (80%)</td>
<td></td>
</tr>
</tbody>
</table>

*one-way ANOVA **Fisher exact test

The mean ages of samples were 63.25 (±6.39), 58.6 (±12.17), 60.2 (±9.93), and 50.9 (±12.6) years for normal, NMIBC, MIBC, and chronic cystitis, respectively. The majority of NMIBC and MIBC patients were male, and most patients with chronic cystitis were female (Table 1). MIBC patients were commonly diagnosed in advanced diseases with a local staging of > T2B and high-grade pathologic differentiation (80%).
The expressions of OPN were significantly higher in MIBC compared to all groups. The expressions of chronic cystitis were lower compared to NMIBC (Figure 1), but there was no significant statistical difference. However, both groups have significantly higher expressions of OPN compared to normal samples (Table 2).

The expressions of OPN were significantly higher in advanced histopathology diseases compared to early diseases while the involvement of nodal metastases and distant metastases had higher expressions of OPN (Figure 2). The results of the independent sample T-tests analyses showed significant differences ($P < .001$).

**DISCUSSION**

OPN has been widely reported to have a central role in the progression of several cancers [18,19,22,26,27]. Since the expression of OPN is elevated in diseased and injured cell sites, it is proposed that OPN attracts macrophages and lymphocytes as host defense mechanisms [28]. In cancer cells, the up-regulation of OPN is correlated with tumor angiogenesis, cell proliferation, chemo-resistance, and cell metastasis [22].

In our study, we found OPN significantly higher in MIBC. However, the expressions of OPN in NMIBC and chronic cystitis had similar results. The role of OPN in inflammation is not fully understood. However, OPN appears to stimulate IL-2 and suppress IL-10 at the sites of inflammation, concomitant with strong stimulation of inflammatory effects. Thus, OPN over-expression is high in the acute phase of leukocyte infiltration [29]. However, the down-regulation of OPN at the wounded area was observed with significantly lower macrophage infiltration and enhanced healing processes [30]. Another study also found that there were correlations of OPN with both tumor staging and aggressiveness [27]. We propose the over-expression in cystitis and NMIBC resulted from different pathways, while another mechanism may involve induration due to tumor enlargement.

As explained above, OPN is considered as a cell defense mechanism. It is reported to have both autocrine and paracrine effects, where the upregulated OPN protects against cytotoxicity through macrophages [31]. This protection is thought to inhibit the production of nitric oxide. Another study showed that OPN null cancer cells injected into mice were eliminated, whereas tumor-derived OPN had more growth and metastases. In our study, MIBC has the highest expression of OPN among groups, and the expression increased concomitant with the aggressiveness of cancer. These results are similar to the findings of Wong et al. [32] and Hussain et al. [33]. Accordingly, suppressing OPN signaling pathways can be conceived to improve the outcome of chemotherapy in bladder cancer.

The applications of OPN approaches in cancer management have been widely used in the last few decades. OPN has been shown to have an important role in angiogenesis, cell proliferation, and invasion by...
multiple pathways; therefore, suppressing this protein can provide promising strategies in treating a variety of cancers [18–22]. Two studies were conducted through OPN pathways, where, in the studies of Tang et al. [26] and Gong et al. [35] the use of RNA interference demonstrated an inhibition, migration, and invasion of gastric cancer. Moreover, by silencing OPN using Lentiviral-OPN-siRNA technologies, it was found that silenced cell lines have fewer detectable cancer cells and metastases, and the treatment outcomes reported longer survival times [34].

Considering the results of these studies, OPN has a significant role in tumor regulation and is associated with chronic inflammation. Several pre-clinical studies have been conducted targeting OPN and have shown promising results. Metformin is commonly used to control diabetes mellitus and treat weight gain. Current research has shown the cytotoxic capability of metformin in both in vitro and in vivo studies, demonstrating its ability to suppress OPN overexpression [36]. Advanced research targeting OPN has the potential to expand our treatment options in bladder cancer. However, meticulously selected patients and strategies are needed due to several reasons. First, while OPN is proved to have immunologic activity, the full impact of OPN alteration in immunotherapies has not been studied yet. Second, OPN is widely expressed in various organs, and it has shown to have a distinct impact. Therefore, we need new insights to overcome and mitigate any adverse effects of these strategies. We are hopeful that new strategies will be developed soon, and significant improvements will be made along these fronts.

This study has several limitations since it was conducted with a limited number of samples and did not completely evaluate the survival data and the outcomes of therapy. However, the data revealed significant differences among groups. Future clinical studies targeting OPN are warranted to improve our management in bladder cancer. In addition, the current trend of management of bladder cancer is encompassed to immunology therapy, the activity of OPN, and CD4+ T-cell gene expression that may indicate the possibility of immune-regulation of OPN. The findings of this study confirmed these findings. We stimulate future research to encompass this peculiar topic.

**CONCLUSIONS**

The expression of OPN was significantly higher in bladder cancer and compared to NMIBC, the OPN expression in MIBC was significantly higher rendering the potential role of OPN expression as a surrogate biomarker marker to determine suitable treatment options for patients with bladder cancer.

**DECLARATIONS**

**Competing of Interest**

I declare that I do not have any competing interests, especially with the study funder.

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**REFERENCES**

A Potential Predictive Biomarker for Bladder Cancer Treatment


