Hepatocellular carcinoma (HCC) is the most common type of liver cancer and the fifth most common malignancy worldwide, ranking as the third leading cause of cancer-related death [1]. The 5-year relative survival rate for HCC is approximately 7% [1]. About half of the 782,500 liver cancer cases newly diagnosed worldwide in 2012 were in China [2, 3]. Infection with hepatitis B and C viruses (HBV and HCV, respectively) is the major cause of hepatocarcinogenesis [4]. Other risk factors include cirrhosis, aflatoxin exposure, hemochromatosis, obesity, diabetes mellitus, and metabolic factors [4]. In addition, the high frequency of late-stage disease, metastasis, de novo tumor formation in the diseased liver [5], high rate of recurrence [6], and aberrant gene expression [7, 8] contribute to poor patient prognosis.

The dysregulation of various genes has been linked to HCC prognosis [9, 10]. We hypothesized that certain gene families are associated with HCC prognosis; a
literature search revealed that only few have been identified [11, 12]. Nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) are cytosolic pattern recognition receptors (PRRs) and include five subfamilies—that is, NLRA, NLRB, NLRC, NLRP, and NLRX. These receptors play an important role in monitoring the intracellular microenvironment and mediating inflammation and pathogen clearance [13]. The NLRC family has five members—that is, NOD1, NOD2, NLRC3, NLRC4, and NLRC5 [13]. NOD1 and NOD2 are important components of the innate immune system that protects organisms from Helicobacter pylori infection [14] and function as pattern-recognition molecules that initiate intracellular signaling pathways in response to pathogen-associated molecular patterns [15]. NLRC3 was identified as a negative regulator of type I interferon and proinflammatory cytokine production [16]. In contrast, the functions of NLRC4 are not well understood [17]. NLRC5 is negative regulator of nuclear factor κB and type I interferon pathways, and is thus important for innate immune system homeostasis [18]. NLRX1, the only NLR localized in mitochondria and the sole member of the NLRX family, was found to stimulate reactive oxygen species production following Shigella flexneri infection [19].

Abnormal inflammation is considered as an indicator of tumorigenesis and malignancy. Four major families of PRR—that is, toll-like receptors (TLRs), C-type lectin receptors, RIG-I-like receptors, and NLRs—have been implicated in cell proliferation, angiogenesis, tissue remodeling and repair, and tumorigenesis [20]. Most studies of PRR signaling in malignancies to date have focused on TLR family members. However, recent studies indicate that NLR family members play a direct or indirect role in cancer cell death, angiogenesis, invasion, and metastasis [21, 22]. The present study investigated the prognostic value of NLRC and NLRX family proteins in HCC.

Material and Methods

Patient information

We used an online resource (http://merav.wi.mit.edu/; accessed February 10, 2017) to identify genes of the NLRC and NLRX families that are differentially expressed between normal liver tissue and primary liver tumors. We then used the online website (http://www.oncolnc.org/; accessed September 2, 2017) and The Cancer Genome Atlas (TCGA), (http://tcga-data.nci.nih.gov/tcga) to obtain information on mRNA expression levels of NOD1, NOD2, NLRC3, NLRC4, NLRC5, and NLRX1 at a 75% cutoff; the results presented here are based in part on data generated by TCGA Research (http://cancergenome.nih.gov/) [23]. Clinical data of 360 patients were also downloaded, including race, gender, age, body mass index (BMI), tumor-node-metastasis (TNM) stage, survival time (days), and survival status.

Gene expression profiles were obtained from an independent dataset (GSE14520) in the National Center for Biotechnology Information Gene Expression Omnibus (GEO) (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE14520, accessed February 15, 2017) database [24]. The dataset contained expression profiles generated from HT Human Genome U133A [24] and HT Human Genome U133A_2 [25] arrays. To avoid a batch effect, we selected a profile from the former array that had more patients (n = 231 HCC patients) than the latter. Furthermore, the GeneMANIA website (http://genemania.org/; accessed February 18, 2017) was used to analyze interaction networks of the two NLR families [26].

Functional enrichment analysis of NLRC and NLRX families

The Database for Annotation, Visualization, and Integrated Discovery (DAVID) v.6.7 (https://david-d.ncifcrf.gov/, accessed February 25, 2017) [27, 28] was used for functional enrichment analyses, including gene ontology (GO) functional analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. The former included biological process (BP) and molecular function (MF) terms; in the latter, no results were returned for NLRC and NLRX families.

Survival analysis

In TCGA database, mRNA expression levels in 360 HCC patients were divided into two groups at a cutoff value of 75%; low and high expression groups comprised 270 and 90 patients, respectively. The same cutoff value was applied to the GEO database in order to ensure a reasonable comparison between the two databases. Median survival time (MST) was used to evaluate the prognosis of HCC patients in TCGA database, whereas overall survival (OS) and recurrence-free survival (RFS) were used to assess that of patients in the GEO database. Sex, age, and TNM stage were adjusted in the Cox proportional hazards regression model in TCGA database, whereas gender, age, HBV infection status, alanine aminotransferase (ALT) status, main tumor size, multinodule status, cirrhosis, alphafetoprotein (AFP) level, and Barcelona Clinic Liver Cancer (BCLC) stage were adjusted in the Cox proportional hazards regression model in the GEO database.

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Joint-effects analysis

Only NOD2 and NLRX1 were statistically significant in TCGA database. We carried out a joint-effects analysis of the combination of NOD2 and NLRX1.

The combination of NOD2 and NLRX1 included group I (high NOD2 and low NLRX1 expression), group II (high NOD2 and high NLRX1 expression), group III (low NOD2 and high NLRX1 expression), and group IV (low NOD2 and low NLRX1 expression).

Sex, age, and TNM stage were adjusted in the Cox proportional hazards regression model according to the combination of genes in TCGA database.

Statistical analysis

Pearson correlation coefficients were used to assess correlations among NOD1, NOD2, NLRC3, NLRC4, NLRC5, and NLRX1 genes. Kaplan–Meier survival analysis and the log-rank test were used to calculate MSTs and P

| Variables          | Patients (n = 360) | % of events | MST (months) | HR (95% CI) | Log-rank P |
|--------------------|-------------------|-------------|--------------|-------------|------------|
| Race               |                   |             |              |             | 0.176      |
| Asian              | 155               | 44 (28.4%)  | NA           |             | Ref.       |
| White+others       | 196               | 78 (39.8%)  | 47           | 1.29 (0.89–1.88) |           |
| Missing             | 9                 |             |              |             |            |
| Gender             |                   |             |              |             | 0.311      |
| Male               | 244               | 78 (32.0%)  | 83           |             | Ref.       |
| Female             | 116               | 48 (41.4%)  | 52           | 1.21 (0.84–1.73) |           |
| Age(year) <60      | 168               | 54 (32.1%)  | 84           |             | Ref.       |
| Age(year) ≥60      | 189               | 70 (37.0%)  | 56           | 1.18 (0.83–1.68) |           |
| Missing            | 3                 |             |              |             |            |
| BMI ≤25            | 193               | 66 (34.2%)  | 82           |             | 0.496      |
| BMI >25            | 137               | 45 (32.8%)  | 71           | 0.88 (0.60–1.28) |           |
| Missing            | 30                |             |              |             |            |
| TNM stage A+B      | 252               | 66 (26.2%)  | 84           |             | <0.001     |
| TNM stage C+D      | 87                | 48 (55.2%)  | 26           | 2.48 (1.71–3.61) |           |
| Missing            | 21                |             |              |             |           |
| NOD1               |                   |             |              |             | 0.197      |
| Low                | 270               | 89 (33.0%)  | 71           |             | Ref.       |
| High               | 90                | 37 (41.1%)  | 50           | 1.29 (0.88–1.89) |           |
| NOD2               |                   |             |              |             | 0.012      |
| Low                | 270               | 82 (30.4%)  | 83           |             | Ref.       |
| High               | 90                | 44 (48.9%)  | 47           | 1.60 (1.11–2.30) |           |
| NLRC3              |                   |             |              |             | 0.043      |
| Low                | 270               | 103 (38.1%) | 54           |             | Ref.       |
| High               | 90                | 23 (25.6%)  | 82           | 0.63 (0.40–0.99) |           |
| NLRC4              |                   |             |              |             | 0.700      |
| Low                | 270               | 92 (34.1%)  | 60           |             | Ref.       |
| High               | 90                | 34 (37.8%)  | 56           | 1.08 (0.73–1.60) |           |
| NLRC5              |                   |             |              |             | 0.277      |
| Low                | 270               | 98 (36.3%)  | 56           |             | Ref.       |
| High               | 90                | 28 (31.1%)  | 60           | 0.79 (0.52–1.21) |           |
| NLRX1              |                   |             |              |             | 0.015      |
| Low                | 270               | 103 (38.1%) | 52           |             | Ref.       |
| High               | 90                | 23 (25.6%)  | 85           | 0.57 (0.36–0.90) |           |

BMI, body mass index; TNM stage, tumor, node and metastasis stage; MST, median survival time; HR, hazard ratio; 95% CI, 95% confidence interval; Ref, reference; NOD= nucleotide-binding oligomerization domain; NLRC= nucleotide-binding oligomerization domain-like receptors family CARD domain containing; NLRX1, nucleotide-binding oligomerization domain-like receptors family member X1; Missing, information of race was unavailable in 9 patients; Missing, information of age was unavailable in 3 patients; Missing, information of BMI was unavailable in 30 patients; Missing, information of TNM stage was unavailable in 21 patients.

Bold value in all the tables were statistically significant (P ≤ 0.05).
Uni- and multivariate survival analyses were performed using the Cox proportional hazards regression model. Hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated with the Cox proportional hazards regression model with adjustment for influential clinical characteristics such as gender, age, HBV infection status, ALT status, main tumor size, multinodule status, cirrhosis, TNM stage, and AFP level. P < 0.05 was considered as statistically significant.

### Table 2. Demography and clinical characteristics of 231 HCC patients in GEO database.

| Variables                  | Patients (n = 231) | Overall survival | Recurrence-free survival |
|----------------------------|-------------------|------------------|--------------------------|
|                            | MST (months)      | HR (95%CI)       | Log-rank P               | MST (months)      | HR (95%CI)       | Log-rank P               |
| Gender                     |                   |                  |                          |                   |                  |                          |
| Male                       | 191               | NA               | Ref.                     | 40                | NA               | Ref.                     |
| Female                     | 30                | NA               | 0.59 (0.34–1.00)         | 40                | NA               | 0.47 (0.29–0.75)         |
| Missing<sup>a</sup>        | 10                |                  |                          |                   |                  |                          |
| Age                        |                   |                  |                          |                   |                  |                          |
| ≤60                        | 181               | NA               | Ref.                     | 46                | NA               | Ref.                     |
| >60                        | 40                | NA               | 0.96 (0.65–1.44)         | 37                | 1.01 (0.73–1.41)       |
| Missing<sup>a</sup>        | 10                |                  |                          |                   |                  |                          |
| HBV-virus status           |                   |                  |                          |                   |                  |                          |
| AVR-CC                     | 56                | NA               | Ref.                     | 30                | NA               | Ref.                     |
| CC+NO                      | 162               | NA               | 0.80 (0.56–1.09)         | 48                | 0.78 (0.59–1.04)       |
| Missing<sup>a</sup>        | 13                |                  |                          |                   |                  |                          |
| ALT                        |                   |                  |                          |                   |                  |                          |
| ≤50U/L                     | 130               | NA               | Ref.                     | 53                | Ref.              |                          |
| >50U/L                     | 91                | NA               | 1.06 (0.78–1.44)         | 40                | 1.25 (0.97–1.61)       |
| Missing<sup>a</sup>        | 10                |                  |                          |                   |                  |                          |
| Main tumor size            |                   |                  |                          |                   |                  |                          |
| ≤5 cm                      | 140               | NA               | Ref.                     | 51                | Ref.              |                          |
| >5 cm                      | 80                | 53               | 1.87 (1.38–2.55)         | 30                | 1.37 (1.05–1.78)       |
| Missing<sup>b</sup>        | 11                |                  |                          |                   |                  |                          |
| Multinodular               |                   |                  |                          |                   |                  |                          |
| Yes                        | 45                | 48               | Ref.                     | 27                | Ref.              |                          |
| No                         | 176               | NA               | 0.59 (0.42–0.84)         | 49                | 0.79 (0.58–1.08)       |
| Missing<sup>a</sup>        | 10                |                  |                          |                   |                  |                          |
| Cirrhosis                  |                   |                  |                          |                   |                  |                          |
| Yes                        | 203               | NA               | Ref.                     | 38                | Ref.              |                          |
| No                         | 18                | NA               | 0.23 (0.09–0.63)         | NA                | 0.50 (0.28–0.89)       |
| Missing<sup>a</sup>        | 10                |                  |                          |                   |                  |                          |
| BCLC stage                 |                   |                  |                          |                   |                  |                          |
| 0+A                        | 168               | NA               | Ref.                     | 58                | Ref.              |                          |
| B+C                        | 51                | 20               | 3.68 (2.66–5.06)         | 18                | 2.84 (2.14–3.77)       |
| Missing<sup>u</sup>        | 12                |                  |                          |                   |                  |                          |
| AFP                        |                   |                  |                          |                   |                  |                          |
| ≤300 ng/ml                 | 100               | NA               | Ref.                     | 49                | Ref.              |                          |
| >300 ng/ml                 | 118               | NA               | 0.60 (0.44–0.81)         | 31                | 0.80 (0.62–1.04)       |
| Missing<sup>a</sup>        | 13                |                  |                          |                   |                  |                          |
| NOD1                       |                   |                  |                          |                   |                  |                          |
| Low                        | 187               | NA               | Ref.                     | 42                | Ref.              |                          |
| High                       | 44                | NA               | 0.97 (0.69–1.37)         | 53                | 0.88 (0.65–1.18)       |
| NOD2                       |                   |                  |                          |                   |                  |                          |
| Low                        | 169               | NA               | Ref.                     | 46                | Ref.              |                          |
| High                       | 62                | NA               | 1.21 (0.86–1.70)         | 40                | 1.12 (0.84–1.50)       |
| NLRX1                      |                   |                  |                          |                   |                  |                          |
| Low                        | 168               | NA               | Ref.                     | 46                | Ref.              |                          |
| High                       | 63                | NA               | 0.74 (0.51–1.08)         | 43                | 1.02 (0.76–1.37)       |

AVR-CC, active viral replication chronic carrier; CC, chronic carrier; ALT, alanine aminotransferase; AFP, alpha fetoprotein; BCLC stage, Barcelona Clinic Liver Cancer; Missing<sup>a</sup>, information of gender, age, ALT, multinodular, cirrhosis was unavailable in 10 patients; Missing<sup>b</sup>, information of main tumor size was unavailable in 11 patients; Missing<sup>u</sup>, information of BCLC stage was unavailable in 12 patients; Missing<sup>q</sup>, information of HBV-virus status and AFP was unavailable in 13 patients. Bold value in all the tables were statistically significant (P<0.05).
statistically significant. Vertical scatter plots and survival curves were plotted using GraphPad Prism v.5.0 (La Jolla, CA). Statistical analyses was performed with SPSS software v.22.0 (IBM, Chicago, IL).

Results

Characteristics of patients in TCGA and GEO databases

Detailed characteristics of the 360 patients in TCGA are shown in Table 1. Race, gender, age, BMI, were not associated with MST. On the other hand, TNM stage, NOD2 and NLRX1 levels showed significant associations with MST ($P < 0.001$; adjusted $P= 0.014$ and 0.011, respectively).

The characteristics of the 231 patients in the GEO database are shown in Table 2. Sex, main tumor size, multinodule status, cirrhosis, BCLC stage, and AFP level were significantly associated with OS ($P = 0.048$, $<0.001$, $0.003$, $0.002$, $0.001$, and $0.001$, respectively), whereas gender, main tumor size, cirrhosis, and BCLC stage were significantly associated with RFS ($P = 0.001$, $0.019$, $0.016$, and $<0.001$, respectively).

Correlation analysis of NLRC and NLRX family mRNA expression levels in TCGA and GEO databases

We calculated Pearson correlation coefficients between NLRC and NLRX families. In TCGA database, NOD1 was correlated with other NLRC family members (all $P < 0.001$) but not with the NLRX family member ($P = 0.541$), except for NRLC4 ($P < 0.001$, $r = -0.09$) (Fig. 1A). Only NOD1,
NOD2, and NLRX1 expression data were available in the GEO database. NOD1 was correlated with NOD2 \((P = 0.001)\) but not with the NLRX family member \((P = 0.164)\); there was also no correlation between NOD2 and the NLRX family member \((P = 0.341)\) (Fig. 1B).

**GO functional annotation analysis of NLRC and NLRX families**

To investigate biological functions of the NLRC and NLRX families, BP and MF were evaluated in the GO analysis (Fig. 1C and D). In the KEGG pathway analysis, DAVID did not identify any associations between NLRC and NLRX families.

**Survival analysis of NLRC and NLRX family mRNA expression levels in TCGA and GEO databases**

The characteristics of patients in TCGA database related to prognosis including age, gender, and TNM stage were analyzed with a multivariate Cox proportional hazards regression model. NOD2 and NLRX1 showed significant associations with MST (adjusted \(P = 0.014\), adjusted HR = 1.64, 95% CI = 1.11–2.44; adjusted \(P = 0.011\), adjusted HR = 0.53, 95% CI = 0.33–0.86, respectively) (Table 3). For patients in the GEO database, characteristics such as gender, age, HBV viral infection status, ALT status, main tumor size,
multinodule status, cirrhosis, AFP level, and BCLC stage were analyzed with a multivariate Cox proportional hazards regression model. *NOD1*, *NOD2*, and *NLRX1* were not significantly associated with OS or RFS (Table 4).

**Analysis of mRNA expression levels in TCGA and GEO databases**

Box plots of the expression levels of six genes were downloaded from an online website (Fig. 2A–F). *NLRC3*, *NLRC5*, and *NLRX1* were highly expressed in normal liver tissue whereas the expression in primary liver tumors was low. Scatter plots of *NOD1*, *NOD2*, and *NLRX1* mRNA expression level in the GEO database revealed that only *NOD1* expression differed significantly between tumor and nontumor tissue (*P* = 0.007; Fig. 2G).

Kaplan–Meier curves of mRNA expression levels in TCGA database at a cutoff of 75% are shown in Figure 3. *NOD2*, *NLRC3*, and *NLRX1* all had significant *P* values at this cutoff value (*P* = 0.011, 0.043, and 0.014, respectively).

Kaplan–Meier curves of mRNA expression levels in the GEO database at 75% cutoff are shown in Figure 4. *NOD1*, *NOD2*, and *NLRX1* did not have significant *P* values for OS and RFS (all *P* > 0.05). Scatter plots of the expression levels of six genes in the TCGA and GEO databases at a 75% cutoff are shown in Figure 5A and B.

**Figure 2.** (A–F) mRNA expression levels of *NOD1* (A), *NOD2* (B), *NLRC3* (C), *NLRC4* (D), *NLRC5* (E), and *NLRX1* (F) genes in normal liver tissue and primary liver tumors. G, *NOD1*, *NOD2*, and *NLRX1* genes in the GEO database.
Joint-effects analysis of NLRC and NLRX family mRNA expression levels in TCGA database

We carried out a joint-effects analysis for the combination of NOD2 and NLRX1. In the joint-effects analysis of the combination of NOD2 and NLRX1, group I had the shortest MST of 38 months (adjusted P = 0.007), whereas group III had the longest MST of 85 months (adjusted P = 0.001, adjusted HR = 0.31, 95% CI = 0.16–0.61) (Table 5). Interaction networks among NOD1, NOD2, NLRC4, NLRC5, and NLRX1 are shown in Figure 5C. Kaplan–Meier survival curves of the analyses of two genes are shown in Figures 5D.

Discussion

In this study, we investigated the association between NLRC and NLRX family genes and HCC. We determined that the mRNA expression levels of these two NLR families are associated with distinct prognoses. Thus, the mRNA expression levels of NLRC and NLRX family genes alone or in combination—especially NOD2, and NLRX1 combined—can predict HCC prognosis.

NLR family genes are known to regulate the formation of the inflammasome and pro-inflammatory chemokines and cytokines that are involved in the host response to pathogens [29, 30]. However, there is little known about the relationship between these gene families and cancer, especially HCC. NOD1 is an important factor in the defense against Pseudomonas aeruginosa [31], Listeria monocytogenes [32], and H. pylori [33] infection and has been linked to Crohn’s disease [34, 35], inflammatory bowel disease [36], and Behcet’s disease [36]. NOD2 was found to be associated with Crohn’s disease [37], ischemic cardiovascular disease [38], Blau syndrome [39], allergic rhinitis [40], and artherosclerosis [41]. NLRC3 is a biomarker for colorectal cancer [42]; NLRC4 was related to enterocolitis [43], recurrent macrophage activation syndrome [44], and familiar cold autoinflammatory syndrome [45]; and NLRC5 has been implicated in chronic periodontitis [46]. NLRX1 was found to be associated with risk of gastric cancer in the Chinese population [47]. Interestingly, the other four genes in the NLRC and NLRX
gene families did not show any direct or indirect associations with HCC, with the exception of \textit{NOD1/NOD2} pathway, which acted synergistically with \textit{NLRP3}.

In this study, we found that \textit{NOD2} was highly expressed in primary liver tumors, which was associated with shorter MST. In contrast, \textit{NLRX1} was expressed at low levels in primary liver tumors, which was also linked to short MST. In the joint-effects analyses, groups I had the shortest MST. In theory, the opposite trend in expression level for each gene should be associated with the best prognosis. Strikingly, this was only observed in group III.

\textbf{AFP is a widely used serum diagnostic and prognostic biomarker for HCC. However, its prognostic value remains controversial.}\n
\textit{Serum AFP levels have been reported as an indicator of OS and RFS in HCC [48, 49]. However, this was not confirmed in other studies [50–52]. Its sensitivity for HCC screening ranges from 41 to 65\% at a cutoff of 20 ng/mL [53–56]. In recent years, various biomarkers have emerged for diagnosing HCC and predicting patient outcome, including glypican 3 and insulin-like growth factor (IGF)II mRNA [57], Keap1 and pNrf2 [58], 3-microRNA and AFP [59], CXCL1 [60], minichromosome maintenance complex -7 [61], and IGF1 receptor [62], among others. Mitochondria release molecules such as cytochrome c and apoptosis-inducing factor into the cytosol [63] and are associated with autophagy [64]. Exogenous substances applied to HCC cell lines can affect the release of these molecules and thereby alter caspase-independent apoptosis signaling (i.e., the mitochondrial pathway) [65]. Mitochondrial NLRX1 expression is altered in liver tissue in HCC, suggesting that it could affect apoptosis in HCC, although the detailed mechanisms remain to be determined.}

\textbf{There were some limitations to our study that need to be recognized. Firstly, larger sample sizes are needed in order to increase the reliability of the findings. Secondly, more clinical data concerning tumor progression and...}
prognosis such as smoking and drinking status, Child–Pugh scoring, presence of cirrhosis, transarterial chemoembolization, antitherapy status, radical resection status, pathological differentiation diagnosis, main tumor size, numbers of tumors, status of tumor capsules, regional invasion, intrahepatic metastasis, and vascular invasion should be included to better evaluate the relationship between the two NLR gene families and HCC. Thirdly, the more commonly used indices of OS and RFS should be applied to the evaluation of HCC prognosis. Fourth, further investigations focusing on functional part needs to be well explored in multi-center, multi-racial countries. And functional validation in a well-designed clinical trial will be further studied in our future researches.

Figure 5. Scatter plots of NOD1, NOD2, NLRC3, NLRC4, NLRC5, and NLRX1 gene expression levels in TCGA (A), GEO (B) databases and gene–gene interaction networks among selected genes constructed by GeneMANIA (C) and survival curves for joint-effects analysis of the combination of NOD2 and NLRX1 genes in TCGA database (D).
Conclusion

Our study demonstrates that NOD2, and NLRX1 may be potential prognostic biomarkers of HCC and their combination showed a strong interaction and better predictive value for HCC prognosis. Due to the small sample size and incomplete clinical information in this study, further well-designed and larger sample size studies are necessary to validate our results.

Conflict of Interest

None declared.

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Table 5. Joint-effects analysis of the combination of NOD2 and NLRX1 in TCGA database

| Group | NOD2 expression | NLRX1 expression | Patients (n = 360) | MST (months) | Crude P | Crude HR (95% CI) | Adjusted P* | Adjusted HR* (95% CI) |
|-------|----------------|-----------------|-------------------|--------------|---------|-----------------|-----------|---------------------|
| I     | High           | Low             | 67                | 38           | 0.005   | Ref.            | 0.007     | Ref.                |
| II    | High           | High            | 23                | 56           | 0.142   | 0.59 (0.29–1.20)  | 0.228     | 1.61 (0.27–1.36)    |
| III   | Low            | High            | 67                | 85           | 0.001   | 0.32 (0.17–0.62)  | 0.001     | 0.31 (0.16–0.61)    |
| IV    | Low            | Low             | 203               | 82           | 0.022   | 0.62 (0.42–0.93)  | 0.043     | 0.63 (0.41–0.99)    |

Adjusted P*, adjustment for gender, age, TNM stage. Bold value in all the tables were statistically significant (P ≤ 0.05).
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