

*Original Article***Clinicopathological significance of E-cadherin,  $\beta$ -catenin and p53 expression in gastric adenocarcinoma**

Mohammad Reza Zalf<sup>a</sup>, Omeed Moaven<sup>b</sup>, Hamid Asadzadeh Aghdaee<sup>a</sup>,  
Kamran Ghafarzadegan<sup>c</sup>, Khadijeh Jami Ahmadi<sup>d</sup>, Mehdi Farzadnia<sup>e</sup>,  
Azadeh Arabi<sup>b</sup>, Mohammad Reza Abbaszadegan<sup>\*b</sup>

**Abstract**

**BACKGROUND:** E-cadherin/catenin complexes exert a role in cell adhesion.  $\beta$ -catenin is a key player in Wnt signaling pathway in gastric cancer. P53 is a tumor suppressor gene which also regulates apoptosis. We assessed the expression of E-cadherin,  $\beta$ -catenin and p53 in gastric adenocarcinoma, and their correlations with clinicopathological features.

**METHODS:** Fifty six formalin-fixed, paraffin-embedded archival specimens of gastric adenocarcinoma were randomly included as cases. Adjacent tumor-free gastric mucosa of different premalignant stages was obtained from the cases. Immunohistochemical staining was performed to assess E-cadherin,  $\beta$ -catenin and p53 expression.

**RESULTS:** All chronic atrophic gastritis and intestinal metaplasia revealed normal membranous staining. Only one patient with dysplasia had abnormal expression of E-cadherin and  $\beta$ -Catenin. Abnormal E-cadherin,  $\beta$ -catenin and p53 expression was found in 50%, 48.2% and 76.8% of cancer specimens respectively. Abnormal expression of E-cadherin was significantly correlated with aberrant  $\beta$ -catenin expression. Abnormal E-cadherin and  $\beta$ -catenin expression were significantly correlated with depth of tumor invasion and advanced gastric cancer ( $p < 0.05$ ), lower degree of differentiation and diffused tumor type ( $p < 0.001$ ). Node metastasis was not influenced by abnormal expression of E-cadherin and  $\beta$ -catenin. P53 was not associated with clinicopathological variables.

**CONCLUSIONS:** Abnormal expression of the E-cadherin and  $\beta$ -catenin were associated with each other and influenced by histogenesis of gastric cancer and malignant behavior of tumor but not significant in premalignant lesions. They are more frequent in diffuse type and associated with advanced gastric cancer. P53 alterations are more frequent in the Iranian population compared with others.

**KEYWORDS:** Gastric Cancer, E-cadherin,  $\beta$ -catenin, p53, Immunohistochemistry.

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**G**astric cancer is the second leading cause of cancer related death in the world. In Iran, it is the most frequent cancer and top of the list of cancer mortality.<sup>1</sup> There are geographical variations in epidemiology and genetics of gastric adenocarcinoma. Japan and China have highest incidence of gastric cancer with different epidemiologic and

genetic patterns as compared with western countries.<sup>2</sup> Similarly, Iran stands above the average in the incidence of gastric cancer (ASR: 26.1 in males and 11.1 for females)<sup>1</sup> but the genetic of gastric cancer and its pathophysiologic implications in this high incidence region of the world and probable variations has remained to be clarified. E-cadherin,  $\beta$ -catenin

<sup>a</sup> Research Center for Gastroenterology and Liver Disease (RCGLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran.

<sup>b</sup> Division of Human Genetics, Immunology Research Center, Avicenna Research Institute, Mashhad University of Medical Sciences, Mashhad, Iran.

<sup>c</sup> Department of Pathology, Omid Hospital, Mashhad University of Medical Sciences, Mashhad, Iran.

<sup>d</sup> Department of New Techniques and Sciences, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.

<sup>e</sup> Department of Pathology, Imam-Reza Hospital, Mashhad University of Medical Sciences, Mashhad, Iran.

\* Corresponding Author

E-mail: abbaszadeganmr@mums.ac.ir

and p53 are amongst the most important genes along the gastric carcinogenesis pathways. Several studies have reported controversial results in different pathophysiological aspects.

Cell adhesion mechanisms have a crucial role in processes such as embryonic development, tissue and organ pattern formation, induction and maintenance of normal tissue architecture.<sup>3,4</sup> In epithelial cells, intercellular adhesion is mediated by E(epithelial)-cadherin. This 120-kd molecule belongs to a family of transmembrane glycoproteins and has an important role in calcium-dependent homophilic cell-cell adhesive interactions. Members of the catenin family ( $\alpha$ -catenin,  $\beta$ -catenin and  $\gamma$ -catenin) are required for the function of E-cadherin. They link the cytoplasmic domain of E-cadherin to the actin cytoskeleton or to other transmembrane and cytoplasmic proteins.  $\beta$ -catenin and  $\gamma$ -catenin bind to E-cadherin, whereas  $\alpha$ -catenin forms the link with actin.<sup>5,6</sup> The interaction between E-cadherin and  $\beta$ -catenin at the adherence junction provides one obvious mechanism by which E-cadherin silencing can disrupt growth signaling and develop tumorigenesis. In addition to its role in regulating cell adhesion,  $\beta$ -catenin is a transcription co-factor in the Wnt (wingless) signaling pathway, and elevated levels of free  $\beta$ -catenin are associated with tumorigenesis in humans.<sup>7</sup> E-cadherin competes with the Adenomatous Polyposis Coli (APC) gene product for  $\beta$ -catenin binding,<sup>8</sup> and APC mutation and methylation is found frequently in gastric adenocarcinomas.<sup>9,10</sup> Wild type APC regulates cellular  $\beta$ -catenin levels,<sup>11</sup> therefore, APC may modulate the interaction between E-cadherin and the Catenins, and affect the function of this complex.

A variety of human cancers exhibit abnormal expression of E-cadherin that correlates with higher pathologic grades and advanced tumor stage.<sup>12-16</sup> Decreased or completely lost E-cadherin expression has been reported in gastric cancer, although results regarding the degree of aberrant E-cadherin expression and its correlations with clinicopathological feature were different and sometimes contradictory.<sup>17-19</sup>

P53 is a tumor suppressor gene which prevents cells with DNA damage from entering S-phase by G1 arrest so that as a check point regulator facilitates DNA repair system. It also promotes apoptosis in mutated cells by stimulating bax synthesis.<sup>20</sup> The wild-type p53 protein has a short half-life and is removed rapidly from the nucleus. On the contrary, mutant p53 with a prolonged half-life accumulates in the nucleus, where it is detectable by immunohistochemistry. Therefore, the accumulation of the p53 protein may be considered abnormal.<sup>21,22</sup>

Although many studies have been conducted targeting p53 expression and its impact on clinicopathological features some controversial results have been reported.<sup>23-25</sup>

In this study, we evaluated the immunohistochemical expression of E-cadherin,  $\beta$ -catenin and p53 in gastric adenocarcinoma, and their correlations with clinicopathological features.

## Methods

### Sample Collection

Archival, formalin-fixed, paraffin-embedded gastric adenocarcinoma tissue samples from 56 gastrectomy tissues were randomly included from 8 high-incidence provinces in Iran. Adjacent tumor-free gastric mucosa of different premalignant steps was obtained from all cases. A section from each specimen block was stained with H&E for histological evaluation and representative blocks were chosen for immunostaining. Quality of preservation was good with no significant autolysis. In addition, normal gastric mucosa adjacent to the tumor was used as an internal positive control. Tumors were classified using the Lauren's classification<sup>26</sup> into intestinal and diffuse type. Intestinal type tumors were graded as well, moderate, or poorly differentiated according to the predominant pattern in tumor. Mixed tumors had elements of both intestinal and diffuse patterns. Tumor staging was in accordance with the unified TNM criteria for gastric cancer.

### Immunohistochemical Staining

Formalin-fixed, paraffin-embedded tissue blocks were cut to 4- $\mu$ m-thick sections for

immunostaining. A standard Streptavidin-biotin indirect immunoperoxidase technique was used. Sections were dewaxed, rehydrated and incubated with 3% hydrogen peroxide to block endogenous peroxidase activity. Antigen retrieval was performed by microwave pretreatment in 0.01M citrate buffer (pH 6.0) for 20min at 750 watt. Subsequently, sections were incubated for 2 hours at 37°C with a mouse monoclonal antibody against E-cadherin (Transduction Laboratories, Lexington, KY, USA) diluted at 1:250, Goat Anti- $\beta$ -Catenin (Research Diagnostic, Inc. NJ, USA) and Antibodies against p53 (clone DO-7, M7001, dilution 1:100) followed by biotinylated link anti-mouse and anti-rabbit immunoglobulin (Dakopatts, Denmark) and Streptavidin-biotin peroxidase complex (Dako LSAB®2system, Peroxidase Kit, Denmark). The peroxidase reaction was visualized with 3,3'-diaminobenzidine tetrahydrochloride, supplemented with hydrogen peroxide. The slides were then counterstained with Mayer's hematoxylin and dehydrated before mounting.

#### *Interpretation of Immunostaining*

Staining was scored independently by two observers and a high level of concordance (90%) was achieved. In case of disagreement, the slides were reviewed and a consensus view achieved. A semi quantitative estimation of membranous E-cadherin and  $\beta$ -catenin expression was made, using a composite score obtained by adding the values of the immunoreaction intensity and relative abundance of the E-cadherin and  $\beta$ -catenin immunoreactive cells, as previously reported. Briefly, the intensity was graded from 0 to 3, with 0 for denoting absent staining, 1 for cytoplasmic distribution, 2 for heterogeneous staining (i.e. when tumors were composed of both normal and abnormally staining areas), and 3 for normal membranous pattern of staining. Because staining patterns often varied within the same tumor, particularly when degree of differentiation varied, the score was based on the dominant pattern. Tumors with more than 10% variation were scored as heterogeneous.

The abundance of positive cells was graded from 0 to 4 (0: less than 5% of positive cells, 1: 5-25%, 2: 26-50%, 3: 51-75%, 4: 76-100%).

E-cadherin and  $\beta$ -catenin expression was considered preserved when the composite score was 6 or 7. Cases with scores between 0 and 5 were considered as decreased expression. In order to facilitate statistical analysis, all tumors with loss of membranous expression were categorized as abnormal including those with absent, heterogeneous or cytoplasmic staining patterns. P53 immunoreactivity was defined by observing nuclear staining. P53 expression was graded as negative (< 20%) or positive (> 20% of tumor cells stained).<sup>27</sup>

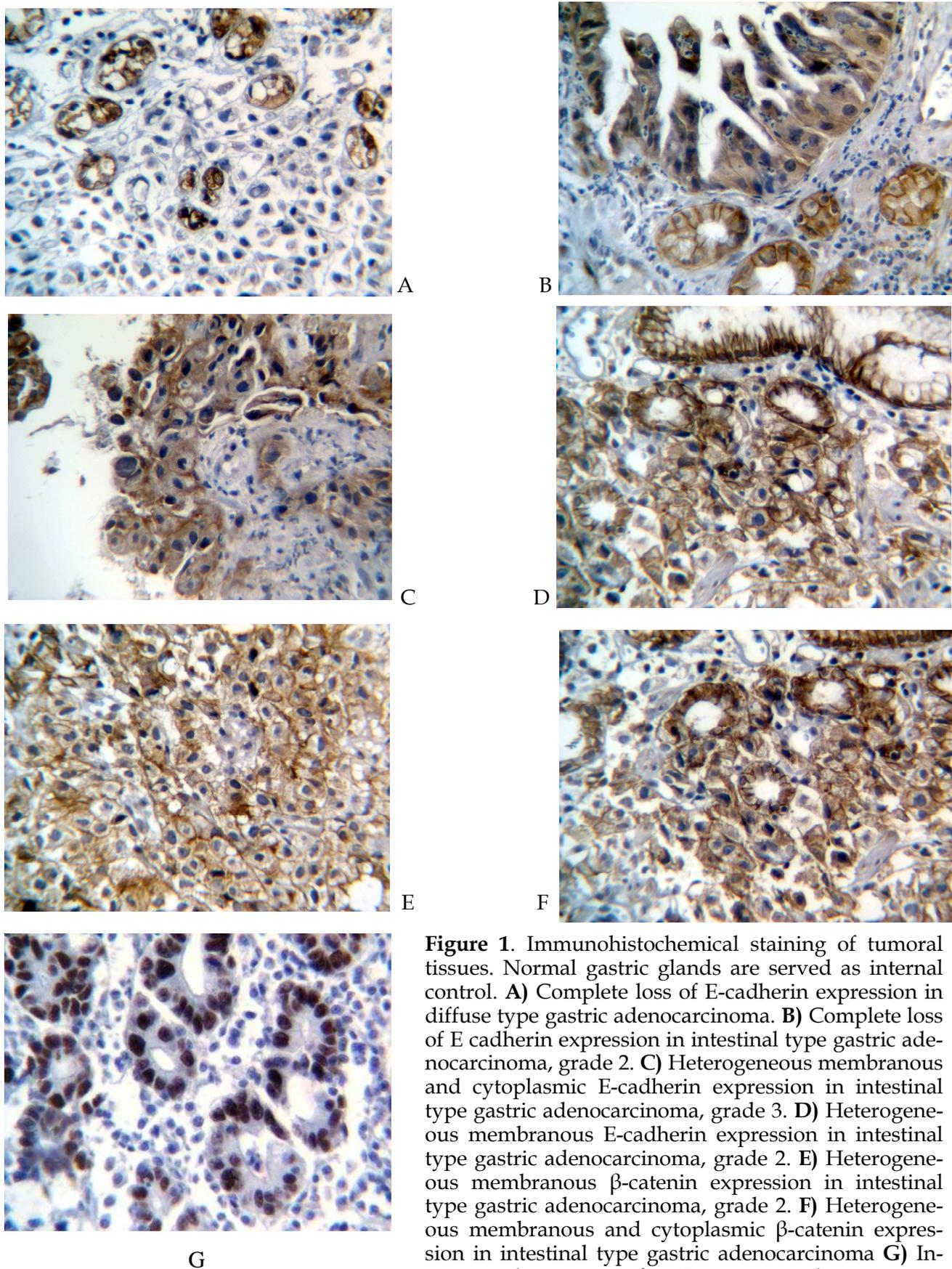
#### *Statistical Analysis*

Statistical analysis was performed using SPSS software (version 11.5). The correlation between two variables was evaluated using Pearson's chi-square and Fisher's exact test. Statistical significance was defined as  $p < 0.05$ .

#### **Results**

Study population was consisted of 40 male and 16 female patients (median age: 60, ranged from 26 to 76) from 8 high-incidence provinces in Iran. In normal histology and pre-cancerous gastric mucosa including atrophic gastritis, intestinal metaplasia E-cadherin was expressed uniformly at the cell membrane. One patient with dysplasia had abnormal expression of both E-cadherin and  $\beta$ -catenin.

Figure 1 demonstrates different patterns of staining. Abnormal E-cadherin,  $\beta$ -catenin and p53 expression was found in 50%, 48.2% and 76.8% of cancer specimens, respectively. Abnormal expression of E-cadherin and  $\beta$ -catenin were significantly correlated with each other with 85.7% of cases showing same pattern of expression. Abnormal E-cadherin and  $\beta$ -catenin expression was associated with depth of tumor and advanced stages ( $p < 0.05$ ) while node metastasis was not influenced by abnormal expression of E-cadherin and  $\beta$ -Catenin. Abnormal E-cadherin and  $\beta$ -catenin expression was significantly correlated with lower degree of differentiation ( $p < 0.001$ ). It was also significantly more frequent in diffused type of



tumor. In mixed-type carcinomas with elements of both intestinal and diffuse patterns, intense staining was seen in the intestinal component whereas areas with a diffuse pattern showed more heterogeneous and abnormal or diminished patterns of staining. P53 was not associated with clinicopathological variables. Expression in neither of genes was affected by gender and age. Table 1 shows clinicopathological features of patients and their correlation to E-cadherin,  $\beta$ -catenin and p53 expression.

## Discussion

Abnormalities in expression and function of E-cadherin,  $\beta$ -catenin and p53 have been shown in various cancers. The aim of this study was to clarify the clinicopathological significance of expression of some important genes in gastric carcinogenesis in the Iranian population. For this purpose, expression of E-cadherin,  $\beta$ -catenin and p53 were assessed. Previous studies have shown variable abnormal E-cadherin expression ranging from 23% up to 92% of tumors.<sup>17,28,29</sup> However different antibodies and different scoring systems of previous studies make the comparison of the overall expression of E-cadherin-catenin complex difficult. Our results showed abnormal E-cadherin and  $\beta$ -catenin expression in 50% and 48% of the gastric carcinomas respectively, consistent with the study performed by Zhou YN et al.<sup>30</sup>

Eighty four percent of tumors exhibited abnormal pattern of expression for E-cadherin and  $\beta$ -catenin simultaneously, significantly correlated with each other, consistent with results of Huiping et al.<sup>31</sup> E-cadherin interacts with either  $\beta$ - or  $\gamma$ -catenins which bridge E-cadherin to the cytoskeleton through  $\alpha$ -catenin.<sup>32</sup> Abnormal expression of  $\alpha$ - or  $\beta$ -catenin due to mutation, deletion, or post-translational modification (e.g. by phosphorylation) may account for loss of cell adhesion in the tumors retaining normal expression of E-cadherin.<sup>33</sup>  $\beta$ -catenin exerts a dual role as an essential cytoplasmic-interaction counterpart of cadherins, essential for cell-cell-adhesion,

and as a nuclear partner of the T-cell factor (TCF)/lymphocyte-enhancer factor (Lef) family of transcription factors regulating genes of the canonical Wnt signaling pathway.<sup>34</sup> Switching from one role to another takes advantage from distinct molecular forms of  $\beta$ -catenin that have different binding properties.<sup>35</sup> The manifestation of cancer by aberrant Wnt signaling pathway most likely results from inappropriate gene activation mediated by stabilized  $\beta$ -catenin. APC mutations and  $\beta$ -catenin gene mutations increase the association of  $\beta$ -catenin with the TCF-4 and Lef-1,<sup>36,37</sup> which are involved in the transcriptional regulation of E-cadherin<sup>38</sup> and possibly, of other target genes and provide evidence of functional interaction between signaling pathways and cell adhesion molecules during tumorigenesis. As an important molecule in cell adhesion, loss of E-cadherin will disrupt proliferation inhibition and lead to more scattered types of tumor like diffuse type which have more malignant behavior with poorly differentiated cells. We demonstrated a significant correlation between E-cadherin expression and tumor histology. Aberrant E-cadherin expression is associated with both poorly differentiated and diffuse-type adenocarcinomas, in accordance with the results of Gabbert et al and Bolk et al.<sup>29,39</sup> Abnormal expression patterns of  $\beta$ -catenin were also more frequent in poorly differentiated and diffuse-type tumors.

Gastric cancer has one of the most complex genetic pathways with lots of questions still remained to be clarified. Controversial conclusions have been reported about the role of E-cadherin in the gastric carcinogenesis. Correa et al have proposed a pathway of carcinogenesis starting with atrophic gastritis, followed by intestinal metaplasia and dysplasia, leading to gastric adenocarcinoma.<sup>40</sup> Although intestinal types are best described by this cascade, diffuse types are not consistent with this proposed pathway.<sup>39</sup> Some previous studies reported E-cadherin alterations as a crucial early events significantly rising in dysplasia.<sup>30</sup> Our result does not support this conclusion in our

**Table 1.** Clinicopathological features of patients and their correlation with E-cadherin,  $\beta$ -catenin and p53 expression

Variable	E-cadherin			$\beta$ -catenin			p53		
	Normal	Abnormal	p	Normal	Abnormal	p	Positive	Negative	p
<b>Total</b>	28	28		29	27		43	13	
<b>Gender</b>									
Male	20	20		18	20		9	31	
Female	8	8	1.000	11	7	0.568	4	12	1.000
<b>Age</b>									
< 60	9	13		7	13		14	6	
> 60	19	15	0.274	22	14	0.094	29	7	0.510
<b>Path. Grade</b>									
1	21	5		18	8		19	7	
2	5	7		6	6		8	4	
3	2	16	< 0.001	5	13	< 0.001	16	2	0.306
<b>Path. Type</b>									
Intestinal	25	10		24	12		25	10	
Diffuse	2	14		4	11		13	3	
Mix	1	4	< 0.001	1	4	< 0.001	3	0	0.324
<b>Depth of Invasion</b>									
Early (T <sub>1</sub> )	8	1		8	1		6	3	
Advanced (T <sub>2-4</sub> )	20	27	< 0.05	21	26	< 0.05	37	10	0.419
<b>Node Metastasis</b>									
N <sub>0</sub>	13	12		15	10		19	6	
N <sub>1-3</sub>	15	16	1.000	14	17	0.269	24	7	1.000

population. Almost all of the preneoplastic lesions exhibited normal expression, in accordance with the results of Chen HC et al.<sup>41</sup> It was also significantly more frequent in advanced gastric cancer and the majority of early gastric cancers preserved E-cadherin expression. We conclude that loss of normal E-cadherin expression affects invasion of tumor to the muscularis propria resulting in advanced gastric cancer. In this study E-cadherin influenced tumor depth of invasion but it was not significantly associated with lymph node metastasis. This reveals that preserved E-cadherin expression does not necessarily lead to intact cell adhesion mechanisms. It might be a result of malfunctioned E-cadherin protein despite normal staining in some cases. Point

mutations or partial methylation might affect the normal function of E-cadherin gene product while the immunostaining is preserved normally.<sup>42</sup>

Varied range of results has been reported for p53 expression in gastric cancer and its correlations with clinicopathological features in several studies. We demonstrated p53 expression in 76.8% of cases, which is more frequent than previous studies ranging from 25.2% to 57.5%.<sup>43-47</sup> Correlation with intestinal type was reported by Hurlimann et al and Fukomaga et al<sup>48,49</sup> while many studies demonstrated no preference of tumor histological type. Joypaul et al and Strazynska et al reported impact of p53 overexpression on staging<sup>22,50</sup>; Kim et al and Roviella et al revealed its association with

tumor depth of invasion and lymph node metastasis.<sup>51,52</sup> Lee et al also showed lymph node metastasis is influenced by p53 overexpression<sup>53</sup> while Deveci et al reported less frequency of p53 expression in the presence of more than five metastatic lymph nodes.<sup>54</sup> We demonstrated that p53 overexpression was not associated with clinicopathological features, in consistent with the results of Sasao et al and Gamboa-Dominguez et al.<sup>55,56</sup>

### Conclusions

In conclusion, loss of normal E-cadherin and  $\beta$ -catenin occurs in a considerable proportion of gastric carcinomas while it is not significant

in the precancerous lesions and p53 is over expressed more frequently in Iranian population. E-cadherin and  $\beta$ -catenin are associated with more malignant behavior of tumor and might be served as a marker of differentiation. Loss of these cell adhesion molecules is associated with diffuse type. It is more common in advance gastric cancer. Clinicopathological features were not influenced by p53 overexpression.

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### Conflict of Interests

Authors have no conflict of interests.

### Authors' Contributions

MRA and MRZ have designed the study. MRA, HAA, KGh, KhJA, MF, AA and OM have performed the study. KGh, MRA and OM have analyzed the results. OM and KJA have written the manuscript. MRA and MRZ scientifically revised the manuscript. All authors have read and approved the content of the manuscript.

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