1. Introduction

Breast cancer, a diverse disease with varied clinical outcomes, poses significant challenges to prognosis and treatment decision-making. [1]. Advancements in understanding the molecular pathways responsible for breast cancer have led to new methods for predicting tumor behavior and treatment responses, as well as evaluation of the tumor's aggressive potential [2,3]. Assessing the presence of a tumor in the axillary lymph nodes metastasis in breast cancer patients has a critical approach in the grading of breast cancer as well as the prognosis [4]. A specific diagnostic approach involves identifying metastatic cells by analyzing RNA transcripts from genes, such as CK19 and MGB, which are highly expressed in breast-origin cells but minimally in others. Furthermore, these genes are found only in small amounts in metastatic non-metastatic tissues. These include cytokeratin (CK19) and myoglobin (MGB), two indicators that have been less investigated in correlation with the enzyme Isocitrate Dehydrogenase 1 (IDH1) gene. The aim of this study was to assess the expression of this gene could have significant effects on the progression of metastasis and invasive disease in breast cancer patients. We used the molecular method of RT-PCR with SYBR-Green to analyze breast tumor tissue from patients with metastasis and non-metastasis, the latter confirmed by the pathology department of Shohada-e Tajrish Hospital (serving as a control group). Also, patients population and its relationship with the degree of tumor in the IDH1 gene was investigated. The IDH1 gene has shown high expression in patients with metastatic breast cancer rather than in patients with non-metastatic breast cancer. The metastatic samples were compared with non-metastatic samples for IDH1 mRNA expression. In this research work, 72.5% (29 samples) were up-regulated in comparison to 27.5% of samples (11 samples) that did not exhibit high expression (P=0.000). This study examined the IDH1 gene expression, suggesting that changes in this gene's expression could impact the prognosis of breast cancer. However, further research is needed to draw definitive conclusions.

Keywords: IDH1 gene, Breast Cancer, Invasive, Correlation.

1. Correlation of IDH1 gene expression error in breast tumor biopsy in patients with invasive ductal carcinoma

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One of the most important cancers in terms of worldwide prevalence is breast tumors, which have been less investigated in correlation with the enzyme Isocitrate Dehydrogenase 1 (IDH1) gene. The aim of this study was that expression of this gene could have significant effects on the progression of metastasis and invasive disease in breast cancer patients. We used the molecular method of RT-PCR with SYBR-Green to analyze breast tumor tissue from patients with metastasis and non-metastasis, the latter confirmed by the pathology department of Shohada-e Tajrish Hospital (serving as a control group). Also, patients population and its relationship with the degree of tumor in the IDH1 gene was investigated. The IDH1 gene has shown high expression in patients with metastatic breast cancer rather than in patients with non-metastatic breast cancer. The metastatic samples were compared with non-metastatic samples for IDH1 mRNA expression. In this research work, 72.5% (29 samples) were up-regulated in comparison to 27.5% of samples (11 samples) that did not exhibit high expression (P=0.000). This study examined the IDH1 gene expression, suggesting that changes in this gene's expression could impact the prognosis of breast cancer. However, further research is needed to draw definitive conclusions.

Keywords: IDH1 gene, Breast Cancer, Invasive, Correlation.
Mutations in IDH1 and IDH2 cause the proteins to gain abnormal functions, converting alpha-ketoglutarate into the cancer-associated metabolite, 2-hydroxyglutarate (2-HG) oncometabolite. Accumulation of 2-HG leads to impaired epigenetic regulation through inhibition of αKGD-dependent histone and DNA demethylases. In addition, Research on small molecule inhibitors targeting mutant IDH1/2 enzymes demonstrates potential in lowering harmful 2-HG levels, offering a promising therapeutic strategy, altering epigenetic regulation disorder, and inducing cell differentiation, have been presented [10].

IDH gene mutations are common in several cancers, including glioma, acute myeloid leukemia, and cholangiocarcina, affecting cancer metabolism and treatment responses. Notably, the neomorphic activity of the mutated IDH creates distinct patterns in cancer metabolism, epigenetic displacement, and therapeutic resistance [11].

Several pieces of evidence show that during the transformation of epithelial cancer cells, they can acquire mesenchymal properties through a process called epithelial-to-mesenchymal transfer (EMT). This process allows cancer cells to spread by increasing their ability to invade and migrate. Discoveries that mutations in metabolic genes, like FH, SDH, and IDH, trigger the EMT process underscore the link between cancer metabolism and cell migration and invasion [12].

Mutations in isocitrate dehydrogenase (IDH1) and IDH2 are among the most common genetic changes in intrahepatic cholangiocarcinoma (IHCC). IDH mutant proteins in intrahepatic cholangiocarcinoma and other malignancies have an abnormal enzymatic activity that allows them to convert alpha-ketoglutarate to 2-hydroxy ketoglutarate, which inhibits the activity of multiple αKG-dependent dioxygenases and leads to changes in cell differentiation. Mutated IDH inhibits hepatic progenitor cell differentiation through 2-HG production and suppression of HNF-4α, a major regulator of hepatocyte identity and inactivity. These studies provide a functional link between IDH mutations, hepatocyte fate, and IHCC pathogenesis [13].

Acute myeloid leukemia (AML), chondrosarcoma, and cholangiocarcinoma are intrahepatic. The mutant protein loses its normal enzymatic activity and acquires a new ability to produce 2-hydroxyglutarate, which is combat to life. 2-HG competitive enzymes inhibit α-KG-dependence cells that play an important role in gene regulation and tissue homeostasis. Mutated IDH expression disrupts cell differentiation at different cell lines and promotes tumor development in association with other cancer genes [14].

Specific mutations in the isocitrate dehydrogenase (IDH) gene were detected in several gliomas, including polymorphic oligodendroglioma and glioblastoma, as well as in leukemia. These mutations produce alpha-ketoglutarate, and 2-hydroxy ketoglutarate (2-HG) distinctly. 2-HG accumulates in very high concentrations, which inhibits the function of alpha-ketoglutarate-dependent enzymes [15]. This phenomenon leads to hypermethylation of DNA and histones and as a result, changes the expression of genes that can activate oncogenes and inactivate tumor suppressor genes [16].

The study of IDH1 gene expression by quantitative assay was performed to achieve the hypothesis that changes in the expression of this gene could have significant effects on the progression of metastasis and invasive disease in breast cancer patients.

2. Materials and methods

We collected 40 invasive ductal carcinoma samples with metastatic symptoms and 40 non-metastatic breast tumor biopsies from Shohada Tajrish Hospital (Tehran, Iran) in June 2021. Data for all patients were saved for analysis. The diagnosis of all breast cancer tumors was confirmed by immunohistochemistry (IHC) staining in the pathology department.

In the classification of samples, two groups of metastatic tumors and non-metastatic tumor samples were considered. The beta-actin gene was used as an internal control for the accuracy of the experiment. Twenty breast tumor specimens that tested negative for metastasis by pathology tests were considered controls. Forty samples of breast cancer tissue with metastasis were studied as a study group in this project.

Tumor severity according to the international standard AJCC-02-TNM was previously classified by the pathologist into groups 1 = 1, 2 = 2a, 3 = 2b, 4 = 3a, 5 = 3b, 6 = 3c, 7 = 4, which in There were Stage 3 and 4 samples for metastatic cases and Stage 1 and 2 for non-metastatic cases, and the grade of the tumor was divided by the pathologist into three categories: I, II, and III, and cases without metastasis were mostly identified with grade 1. In addition to the expression and non-expression of genes, the expression of these genes was also examined by increasing and decreasing the degree and severity of the tumor. The mean age selected was 50 years in the study of tumor samples that did not have tumor metastases.

2.1. Total RNA isolation and cDNA synthesis

RNA was isolated from invasive ductal carcinoma samples by homogenization in 1 mL of Tripure reagent, using approximately 50 μg of each breast tumor specimen. Following homogenization, the mixture was incubated at room temperature for 5 minutes, then 0.2 mL of chloroform was added, mixed thoroughly, and centrifuged at 12,000 x g for 15 minutes at 4°C. RNA was precipitated and added isopropyl alcohol. The upper aqueous phase was transferred to a new tube and centrifuged at 12,000 x g for 10 minutes at 4°C. The RNA pellet was then washed twice with 1 mL of 70% ethanol. The RNA pellet was resuspended in DEPC-treated water to achieve a final concentration of 0.5 μg/μL.

2.2. Reverse transcription and Real-time PCR

or reverse transcription, 5 μg of total RNA was mixed with 0.5 μg of oligo(dT) and 16 μL of RNase-free water, then incubated at 64°C for 10 minutes. Total RNA extracted. The primers for SYBR Green real-time PCR were designed specifically for the IDH1 gene and for the ACTB gene (β-actin) as an internal control, Table 1. The assays were repeated in their entirety for each measurement.

Reverse Transcription is carried out with the SuperScript First-Strand Synthesis System for RT-PCR. The following procedure is based on Invitrogen’s protocol (total RNA 5 g, random hexamers (50 ng/μL) 3 μL, 10 mM dNTP mix 1 μL, DEPC H2O to 10 μL). Incubate the samples at 65°C for 5 min and then on ice for nearly 1-1.5 min. Prepare reaction master mixture. For each reaction (10x RT
buffer 2μl, 25 mM MgCl2 4 μl, 0.1 M DTT 2 μl, RNAase outing 1 μl). Add the mixture to the RNA/primer and then place at room temperature for 3 min. Add 1 μl (50 units) of Superscript II RT to each tube, mix, and incubate at 25°C for 10 min. Incubate the tubes at 42°C for 50 min, heat inactivates at 70°C for 15 min and then chill on ice. Add 1 μl RNase H and incubate at 37°C for 20 min. Store the 1st strand cDNA at -20°C until use for Real-time PCR.

The protocol and design of the study have been approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences with the reference code of IR.SBMU.RETECH.REC.1401.636. Written and signed consent was obtained from each patient or their first-degree relatives after comprehensive explanation was given about the aims and procedures of the study.

3. Results
Breast cancer is a heterogeneous disease that has different pathological and cytological features. The cytogenetic and molecular diagnosis is vital for the prognosis and treatment of this cancer. The application of molecular markers such as IDH1 Gene made the prognosis much more accurate which can predict the metastasis potential [17]. Regarding different studies, based on microarray analysis on breast cancer samples, they found that several genes showed up-regulation.

3.1. RT-PCR results
RT-PCR was conducted on the tumor samples using the protocol described previously. Data analysis was performed using REST and SPSS version 20, incorporating t-tests and Pearson's chi-square tests.

3.2. REST Analyses
IDH1 expression was significantly upregulated in the tumor group compared to the control group, with a mean fold change of 2.711 (standard error = 0.542; P < 0.001). Figure 3. Tables 2 and 3. Also, the graph of relative expression for the IDH1 gene is shown in Figure 3a related to the upregulation of IDH1 in metastatic samples. Also, Figure 1 represents the interquartile range or the middle 50% of observations. The dotted line represents the median gene expression. IDH1 and ACTB amplification graph as Figure 2 A-D.

3.3. Statistical Analyses
Using SPSS for analysis, we compared IDH1 mRNA expression between metastatic and non-metastatic samples. This analysis revealed that 72.5% of the samples

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Table 1. Primer design with gene runner and NCBI BLAST for B-Actin and IDH1 genes.

<table>
<thead>
<tr>
<th>Genes</th>
<th>Forward primer 1</th>
<th>Reverse primer 1</th>
<th>product length</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homo sapiens IDH1, mRNA</td>
<td>TAGGTCGTAGTGGGCTATGGGG</td>
<td>CACCACACCTTCAAG</td>
<td>144</td>
</tr>
<tr>
<td>Homo sapiens actin beta (ACTB), mRNA</td>
<td>GAGAAGATGACCAGACGTC</td>
<td>CACGATGCCAGTGGTACGG</td>
<td>108</td>
</tr>
</tbody>
</table>

Table 2. Relative expression report.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Type</th>
<th>Reaction Efficiency</th>
<th>Expression</th>
<th>Std. Error</th>
<th>95% C.I.</th>
<th>P(H1)</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDH1</td>
<td>TRG</td>
<td>0.9112</td>
<td>2.884</td>
<td>0.542 - 14.701</td>
<td>0.064 - 99.492</td>
<td>0.000</td>
<td>UP</td>
</tr>
<tr>
<td>ACTB</td>
<td>REF</td>
<td>0.8664</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P (H1) - Probability of alternate hypothesis that difference between sample and control groups is due only to chance.

IDH1 is UP-regulated in the sample group (in comparison to the control group) by a mean factor of 2.884 (S.E. range is 0.542 - 14.701). The IDH1 sample group is different from control group. P (H1) =0.000, TRG – Target. REF – Reference.
Table 3. Fold change report.

<table>
<thead>
<tr>
<th></th>
<th>FC Metastatic samples</th>
<th>FC Non-Metastatic samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of values</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>25% Percentile</td>
<td>0.0006250</td>
<td>0.0002000</td>
</tr>
<tr>
<td>Median</td>
<td>0.001200</td>
<td>0.0003000</td>
</tr>
<tr>
<td>75% Percentile</td>
<td>0.003125</td>
<td>0.001075</td>
</tr>
<tr>
<td>Mean</td>
<td>0.004260</td>
<td>0.002675</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>0.01003</td>
<td>0.01129</td>
</tr>
<tr>
<td>Std. Error of Mean</td>
<td>0.001586</td>
<td>0.001785</td>
</tr>
</tbody>
</table>

Table 4. SPSS assessment.

<table>
<thead>
<tr>
<th>Exp. IDH1</th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>negative</td>
<td>11</td>
<td>27.5</td>
<td>27.5</td>
<td>27.5</td>
</tr>
<tr>
<td>positive</td>
<td>29</td>
<td>72.5</td>
<td>72.5</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

3.4. Graph Pad Prism Analyses and T-test Graphs

Analysis using GraphPad Prism revealed a statistically significant difference in IDH1 gene expression between tumor and normal samples ($P < 0.001$), Figure 3b.

In examining the relationship between IDH1 gene expression in metastatic cancer samples, nearly 50% (20 samples) were in grade 2 and 50% (20 samples) were in grade 3, of which 13 grade 2 samples showed an increase in IDH1 gene expression (65%), and 17 grade 3 samples also showed upregulation for this gene, in other words, 32.5% of the upregulated items were in grade 2 and 42.5% of the upregulated samples belonged grade 3 groups. About Stage, 50 % of the samples (10 samples) were in Stage 2, and 95% of the samples (19 samples) were in Stage 3, of which 25% of the Upregulated samples were in Stage 2 and 47.5% of the total upregulated genes related to stage 3. A significant association was found between tumor grade and IDH1 expression ($P = 0.032$), indicating that IDH1 expression tends to increase with higher tumor grades. Also, in examining the significance of the relationship between stage increase in patients with increased expression of the IDH1 gene by Pearson Chi-square test, a significant relationship was found ($P = 0.000$) (Table 5) and The ROC curve analysis demonstrated high specificity and sensitivity of IDH1 expression in distinguishing between metastatic and non-metastatic samples, as illustrated in Figure 4.

4. Discussion

IDH1, an enzyme integral to the Krebs cycle, catalyzes the conversion of isocitrate to alpha-ketoglutarate, playing a vital role in cellular metabolism. Therefore, paying attention to this enzyme and its gene can help us understand cell function. It is not far-fetched that the study of this gene in cancer patients could be very useful in terms of its high prevalence, the costs imposed on patients and many other cases, and the close relationship between cancer and cell metabolism. In this regard, studies have been conducted by researchers on this enzyme and its genes [18].

For example, a study by Lenny Dang et al found that different mutations in the IDH1 enzyme gene are a common feature of early human brain cancers. These mutations occur in an amino acid residue at the active site of the enzyme IDH1 and convert arginine 132 to histidine, which changes the activity of the enzyme instead of producing alpha-ketoglutarate from isocitrate, producing 2HG (2-hydroxyglutarate). It is made from isocitrate. The production of 2-HG, which is known as an oncomtabolic, in this type of mutation causes the increase and accumulation of this substance in the body, which in turn contributes to the formation and malignant development of glioma [19]. A similar study on glioma was conducted by Hao Chen et al., Which shows that the 2HG product produced by the
Hence, the observed alterations in IDH1 expression hold potential for future metastasis prediction studies, both in larger cohorts and across different cancer types.

**Competing interests**

The ethics code is [http://ethics.research.ac. IR.SBMU. RETECH.REC.1401.636](http://ethics.research.ac. IR.SBMU. RETECH.REC.1401.636) in the medical research committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran.

**Conflict of interest statement**

The authors declare no conflict of interest.

**Consent for publications**

The author read and approved the final manuscript for publication.

**Ethics approval and consent to participate**

No human or animals were used in the present research.

**Informed consent**

The authors declare not having any patients in this research.

**Availability of data and material**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Authors' contributions**

Behzad Rostami, Muzi Chen, Neda Mansouri and Aboolfazl Movafagh designed the study and performed the experiments, Neda Mansouri, Aliashgar Keramatinia, Abdul Rahim Nikzamir and Saeed Karima collected the data, Rezvan Ghadyani and Seyed Jalil Hoseini analyzed the data, Behzad Rostami, Muzi Chen, Batool Ghorbani Yeka, Sepehr Kahrizi and Aboolfazl Movafagh prepared the manuscript. All authors read and approved the final manuscript.

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None

**References**


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**5. Conclusion**

IDH1 gene has shown high expression in patients with metastatic breast cancer rather than in patients with non-metastatic breast cancer. Hence, the observed alterations in IDH1 expression hold potential for future metastasis prediction studies, both in larger cohorts and across different cancer types.

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