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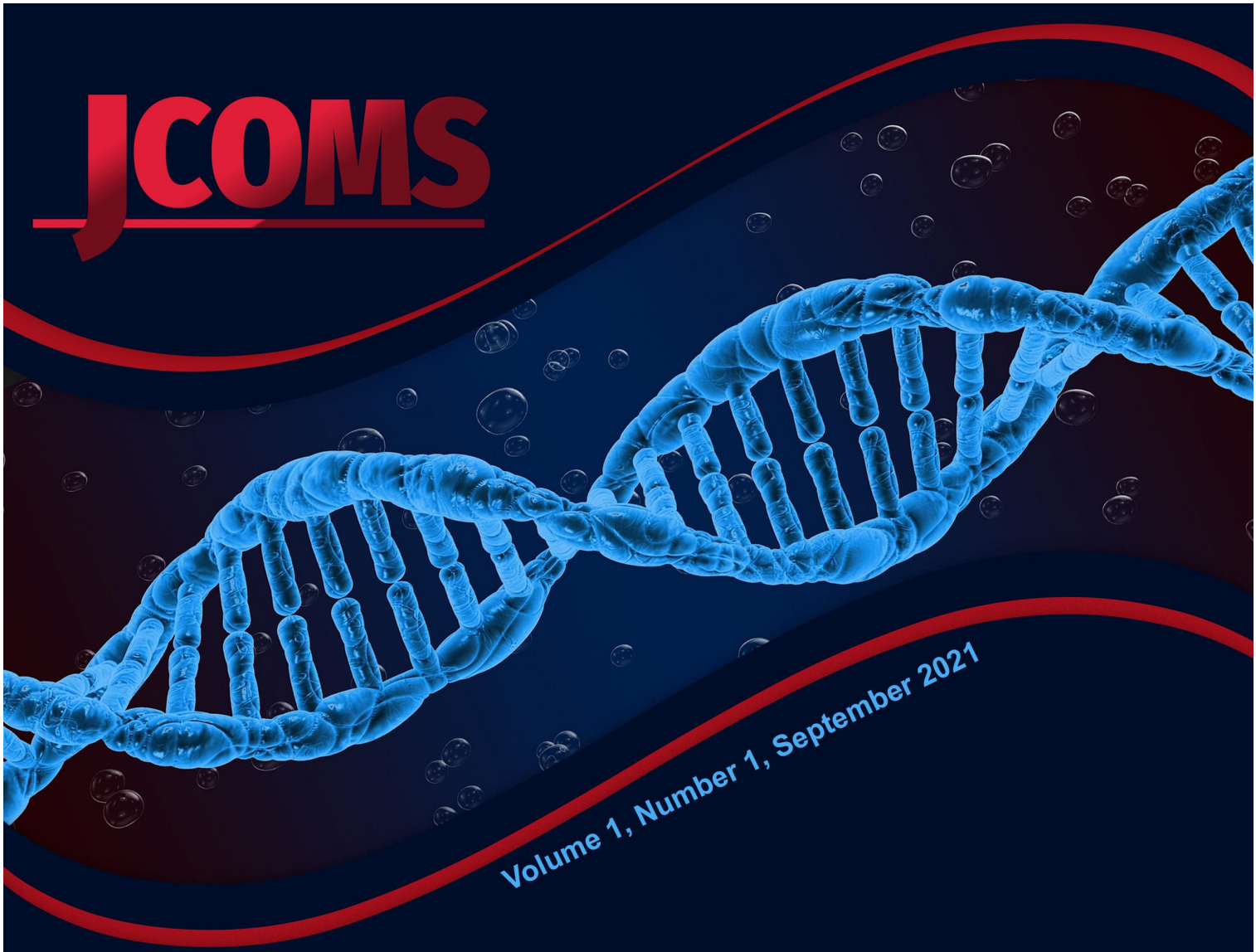

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Genetic alterations of *CDX1*, *CYLD* and *CDKN2B* genes in CRC

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Abstract

Introduction: Colorectal cancer (CRC) is the third most frequent type of cancer in the world. In this explanation, genetic variation is associated in all cancers, particularly CRC, and modifications of numerous genes, such as *CDX1*, *CYLD*, and *CDKN2B*, are linked to tumorgenesis in CRC. As a result, this research was conducted in order to determine changes in the expression of these genes.

Materials and Methods: Speciments of CRC from 72 individuals with confirmation of pathology report,were provided and bought from the Bio banks. Real-time PCR was used to examine the expression of *CDX1*, *CYLD*, and *CDKN2B* genes in tumoral and non-tumoral tissues. These genes' histological associations with grading and staging for upregulation and downregulation were examined.

Result: The expression of *CYLD* (P = 0.01) and *CDKN2B* (P = 0.02) were upregulated significantly, but the *CDX1* (P = 0.03) gene expression was decreased. Correspondingly, there was no significant association between *CDX1* downregulation and *CDKN2B* upregulation with grade, stage, lymph-node metastasis (P= 0.02) and distant metastasis. Moreover, the *CYLD* expression was also significantly associated with high grade (P = 0.03), high stage (P = 0.03), lymph-node metastasis (P= 0.05) and distant metastasis (P= 0.05).

Conclusion: The upregulation of *CYLD* and *CDKN2B* genes and downregulation of *CDX1* gene in tumoral tissues were impressive. Conclusively, the alteration of these genes expression can be considered as a colorectal cancer biomarker.

Keywords: Colorectal cancer, *CDX1*, *CYLD*, and *CDKN2B* genes, Alterations

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Introduction

Colorectal cancer (CRC) is one of the most important causes of cancer mortality in the world (1). The major factor of CRC is the presence of polyps in the colon and also the changes of adenoma to carcinoma process. CRC is the growth of cancer cells in the colon part caused by uncontrolled growth of cells that can proliferate in other tissues irregularly (2). In this way, the term survival of patients with CRC has not been improved in a therapeutic manner. Strongly, there is a vital and emergency requirement for a better understanding in the molecular pathogenesis of CRC in order to recognize the novel biomarkers for prognosis and diagnosis of CRC (3). Correspondingly, molecular genetic methods especially based on DNA and RNA investigating are really practical and useful in diagnostic medicine (4).

CDX1 (caudal-type homeobox 1) is a transcriptional factor and controls enterocyte differentiation in the colon, where its expression is different from the crypt-base stem cell structure. Remarkably, *CDX1* is also a keyword to the capacity of a CRC cell line in differentiation, and it is classified as a negative marker of CRC stem cells. *CDX1* is required for the actual development of the homeostasis of the intestinal epithelium and also intestinal tract (5). Interestingly, *CDX1* is involved in the modulation of a variety of processes comprising cell adhesion, columnar morphology, proliferation, and apoptosis. *CDX1* is a primary controller of enterocyte differentiation and its expression is vital for the transcriptional regulation of a large number of intestine-specific genes essential for the maintenance of the intestinal phenotype, differentiation, and intestine development. Many markers in the differentiation process, containing villin and cytokeratin 20, have been indicated to be directly transcriptionally regulated by this gene. Many evidence indicates the loss or down-regulation of *CDX1* expression in colon cancer tumors and cell lines (6, 7).

Another important gene in gastrointestinal cancers particularly CRC, is the cylindromatosis (*CYLD*) gene, which was initially explored as a tumor suppressor mutated for familial cylindromatosis (8). In addition to skin tumors caused by *CYLD* loss, decreased *CYLD* expression has been described in several types of human cancers comprising breast cancer, hepatocellular carcinoma, cervical cancer, renal cell carcinoma, lung cancer, gastric cancer and also colon cancer. Remarkably, the expression profile and clinical significance of *CYLD* in patients with a series of co-colorectal lesions are so important (9-11).

CYLD was recognized identified as a gene mutated in familial cylindromatosis (FC), a genetic case that predisposes patients for the progression of skin tumors, termed cylindroma. Cylindromas are benign tumors that emerge on the scalp and interestingly is to be derived from hair follicles of stem cells (12). The cylindromatosis patients possess heterozygous germ-line mutations in the *CYLD* gene, but the wild-type *CYLD* allele undergoes loss of heterozygosity (LOH) and rarely somatic mutations in different tumors as tumor suppressor gene. The human *CYLD* gene is situated on chromosome 16q12.1 and encodes a protein of 956 amino acids. The C-terminal region of *CYLD* includes a catalytic domain with sequence homology to USP family members (9, 13). The second important gene is *CDKN2B* which is referred to the *CDKN2A* tumor suppressor gene in a region at 9p21 and this gene is regularly mutated and omitted in many different tumors. Considerably, this gene encodes a cyclin-dependent kinase inhibitor, and it is considered as *CDKN2B* protein, which is a cell cycle regulator (14). The *CDKN2B* gene encodes for *CDKN2B*, which is a member of the *INK4* class of cell cycle inhibitors. Noticeably, *CDKN2B* has ankyrin repeats that permit it to bind and interact of cyclin-dependent kinase *(CDK) 4/6* with cyclin D, through inhibiting the function of *CDK4/6*. Given the critical role of *CDK4*/6 and cyclin D in improving development through the G1 checkpoint, *CDKN2B* performs as a significant inhibitor of cell cycle and cell proliferation (15, 16).

Materials and Methods

Samples collection

The research was performed on 72 patients (53 female and 19 male) which was confirmed by the pathology department and also an agreement by patients. The histopathological status of patients is shown in Table 2. 72 tumoral and 72 non-tumoral (margins tissues) were provided and bought from the Bio banks. In this way, DEPC (diethylpyrocarbonate) was employed to clean and treat all sampling instruments during providing the biopsies (tumoral and nontumoral tissues) in order to avoid RNAs enzyme. Correspondingly, after sampling operation, all specimens were transferred to liquid nitrogen for deep freezing. Vitimately, tissue samples

were stored at − 80 °C for long preservation and study. RNA isolation from human tumoral and nontumoral tissues was performed using a commercial reagent, Trizol (Invitrogen cat no 15596-025, USA.) Less than 1cm of each tissue was crushed in order to powder them by a mortar and pestle in the presence of liquid nitrogen, and 40– 80 mg of powdered tissue was used for RNA isolation according to the manufacture's protocol. RNA quantity was measured by A260/A280 ratio using NanoDrop spectrophotometer (TC100, USA) and also controlled by electrophoresis on agarose gel 2% in order to observe all RNA bands (5S, 18S and 28S).

Relatively, cDNA synthesis was done in the presence of 1 pg total RNA, 4 µL 5X reaction buffer, 10 mM each of dNTPs, and 1 µL (200 U/ µL) by QuantiTect Reverse Transcription Kit (cat no 20S313, USA) in a final volume of 20 µL, by 60 min incubation at 44°C. Meanwhile, Real-time PCR was done on Exicycler q6, Bioneer, USA by using a universal reverse primer and Universal Taqman-specific probe and also the expression levels of all these genes were normalized against GAPDH, RNA as control. The 20 µL PCR comprised 1µµL RT yeild, 0.25 mM universal-specific probe, 0.5 mM each forward and reverse primers. The PCR reagents were all from Qiagen HotStarTaq reagent set (Qiagen, cat no 203205). The mixtures were incubated at 96 °C for 5 min, followed by 43 cycles of 90 °C for 45 s, and 63 °C for 1 min. All reactions were done in triplicate. The CTs were described as the fractional cycle number.

The primers were designed by Allel ID version 7 software. The first cDNA strand was synthesized. The sequences of forward and reverse primers used are given in Table 1. The Real-time PCR tests were accomplished in a Step one instrument (Applied Biosystem, USA) using cDNA. An amount of 1 µl cDNA from each sample was determined for amplification. GAPDH (glyceraldehyde 3-phosphate dehydrogenase) was employed as a housekeeping gene. Amplification occurred in a 20 µl final volume by initial incubation at 96 °C for 5 min, followed by 43 cycles of 95 °C for 30 s and 60 °C for 1 min. The range of up-regulation or down-regulation in each sample was measured using the $2^{-\blacktriangle\blacktriangle ct}$ method.

Table 1. Sequences of primers employed for Real-time PCR action.

	Primer sequence (5′–3′)
Forward *CDX1*	5´-AAGCCTCCGRRCCGCGAATCA-3´
Reverse *CDX1*	5'-GGAAGACTCGTGTATGTATGTGY ATATGTG-3'
Forward *CYLD*	5'-ATGGATAACCCTATTGGCAACTG-3'
Reverse *CYLD*	5'-GTATCCAGTGCTGCGACCGT-3'
Forward *CDKN2B*	5'- TGGCCGGAGGTCATGATG -3'
Reverse *CDKN2B*	5'- GGGCAGCATCATGCACCG -3'

Statistical Analyses

All the acquired data from Real-time PCR were analyzed by exercycle set. Correspondingly, the significant difference was statistically interpreted by paired Student's t-test. $P < 0.05$ was considered statistically significant. Analyses were accomplished using commercially available statistical software (SPSS Statistics software, version 25, Chicago).

Results

Gene expression evaluation in tumoral tissues

The analysis of expression levels of tumoral and corresponding non-tumoral tissues for *CDX1*, *CYLD* and *CDKN2B* genes indicated that the *CYLD* and *CDKN2B* were down regulated in tumoral tissues in comparison with their non-tumoral counterparts ($P = 0.02$). On the contrary, *CDX1* expression level had decreased significantly in 70% of samples (Figure 1,2,3).

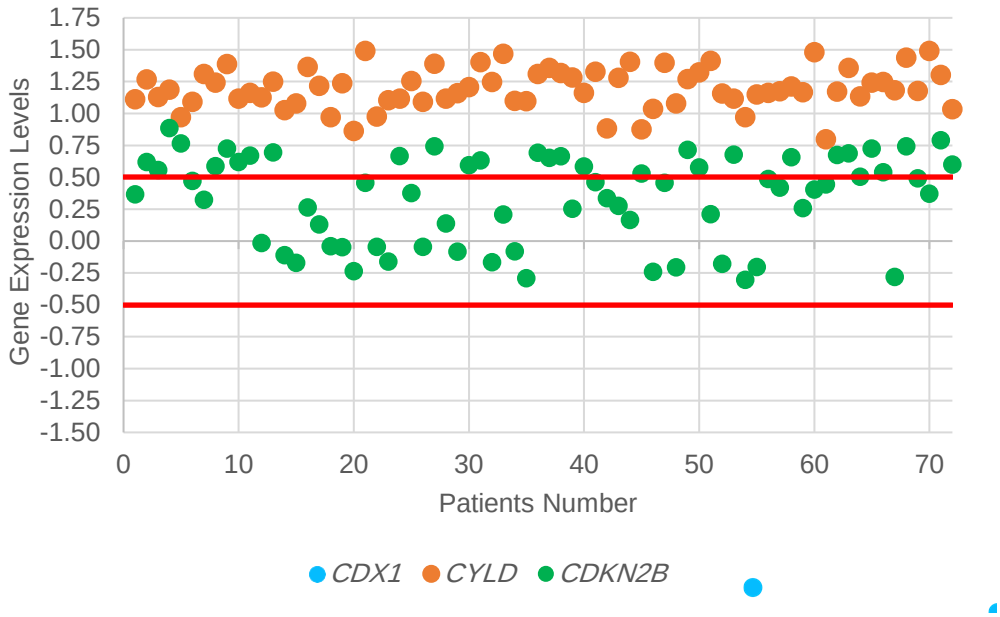


Figure1. Scatter plot analysis of relative expression of *CDX1*, *CYLD* and *CDKN2B* in colorectal cancer patients. The Y-axis indicates the logarithm of relative gene expression. Horizontal red lines represent cut-off values logarithms for two-fold changes in expression (FC≥2.0, p<0.05). The upper part of the graphs indicates up-regulation in the tumoral compared to the non-tumoral tissue; the lower part of the graph indicates down-regulation in the tumoral compared to the non-tumoral tissue (differences in expression ≥ 2; P < 0.05). The *CYLD* (P = 0.01) and *CDKN2B* (P = 0.02) expression level had increased and *CDX1* (P = 0.03) expression level had decreased significantly in tumoral compared to the non-tumoral samples.

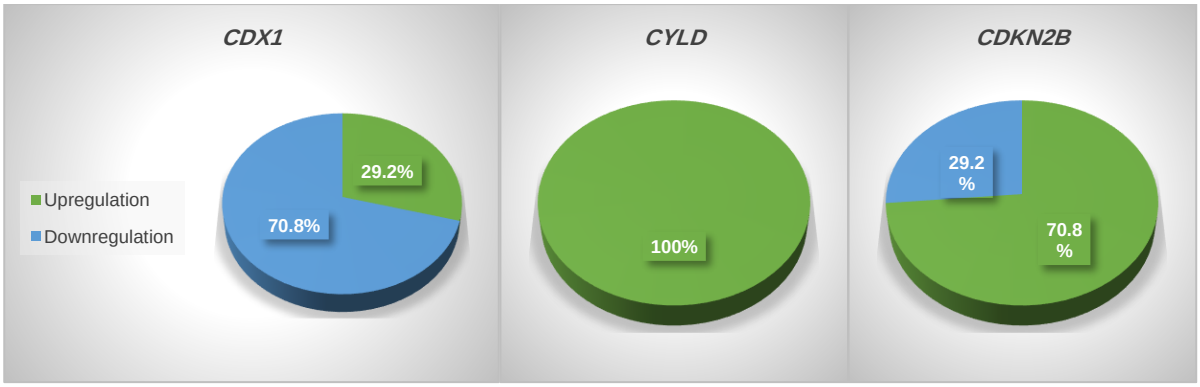
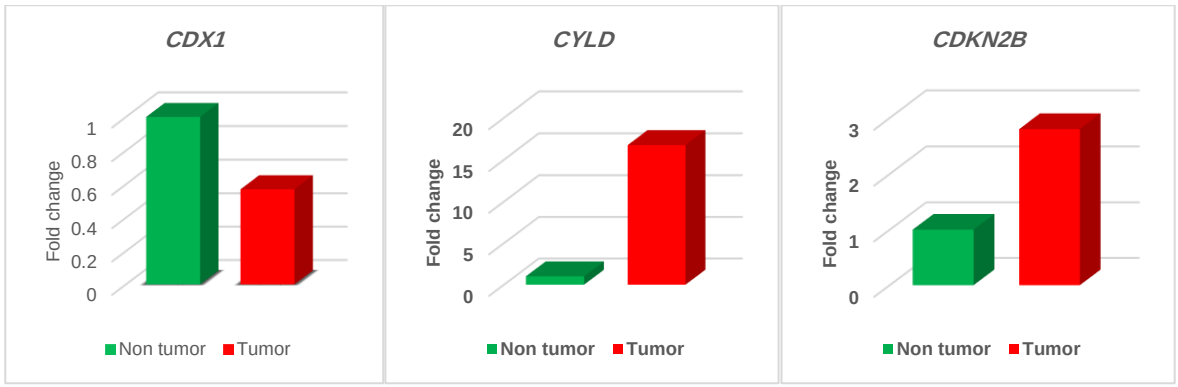


Figure 2. The *CYLD* and *CDKN2B* were down-regulated in tumoral tissues in comparison with their non-tumoral counterparts (P < 0.05).



(a) **(b)** **(c)**

Figure 3. Fold change of **(a)** *CDX1* (P= 0.05), **(b)** *CYLD* (P= 0.02) and **(c)** *CDKN2B* (P= 0.04) expression in tumoral tissues in comparison with non-tumoral (tumor margin) tissues.

Clinicopathological analysis

Clinicopathological consequences of *CDX1*, *CYLD* and *CDKN2B* genes expression were evaluated in 72 patients diagnosed with adenocarcinoma of the colorectal. Patients' clinicopathological characteristics are summarized in Table 2. The analysis of different clinicopathological variables and genes expression correlation is presented in Table (up/down). The mean age of patients was 58.9±12.5 years at the time of diagnosis (female to male ratio, 4:1; age range, 37–88 years). In general, more than half of the patients had advanced stage (Stages III–IV), and high-grade histology. Lymph node metastasis and distant metastasis were observed in more than 60% of the patients.

Table 2. Clinicopathological characteristics of colorectal cancer cases.

Characteristics	Total (N=72) Patients (%)
Gender	
Female	53 (73.6)
Male	19 (26.4)
Age	
< 60 years	38 (52.8)
≥ 60 years	34 (47.2)
Stage	
I	6 (8.3)
II	24 (33.3)
III	38 (52.8)
IV	4 (5.6)
Grade	
Well differentiated	4 (5.6)
Moderate differentiate	26 (36.1)
Poorly differentiate	39 (54.1)
Undifferentiated	3 (4.2)
LM	
Yes	45 (62.5)
No	27 (37.5)
DM	
Yes	44 (61.1)
No	28 (38.9)

The number of gene expressions of all samples was compared and investigated with the stage, grade, lymph node metastasis and distance metastasis of all patients. The analysis of different clinicopathological variables and genes expression correlation is presented in Table 3. Statistical analyzes were performed using SPSS 25 and also Chi-Square test and T-test.

The expression of *CDX1*, *CYLD* and *CDKN2B* was matched with different clinicopathological data of the colorectal cancer patients (summarized in Table 2). There was no significant association between *CDX1* downregulation and *CDKN2B* upregulation with the grade, stage, lymph-node metastasis (P= 0.02) and distant metastasis. Moreover, the *CYLD* expression was also significantly associated with high grade (P = 0.03), high stage (P = 0.03), lymph-node metastasis (P= 0.05) and distant metastasis (P= 0.05) (figure 4, 5, 6).

Table 3. The association of genes expression with clinicopathological qualification. LM: Lymph node Metastasis, DM: Distance Metastasis; ↓/−: decrease or no change of expression; ↑: increase of gene expression

	CDX1		P value	*CYLD*		P value	*CDKN2B*		P value
Tumor Stage	↓/−	↑		↓/−	↑		↓/−	↑	
I-II	18	12	0.7	0	30	0.03	12	18	0.5
III-IV	33	9		0	42		7	35	
Tumor Grade									
I-II	19	11	0.1	0	30	0.03	13	17	0.6
III-IV	30	10		0	42		6	36	
LM									
Yes	30	14	0.4	0	44	0.05	24	22	0.3
No	21	7		0	28		11	15	
DM									
Yes	32	12	0.5	0	44	0.05	21	23	0.2
No	19	9		0	28		15	13	

LM: Lymph node Metastasis, DM: Distance Metastasis

The Association of *CDX1*, *CYLD* and *CDKN2B* expression with clinicopathological qualifications

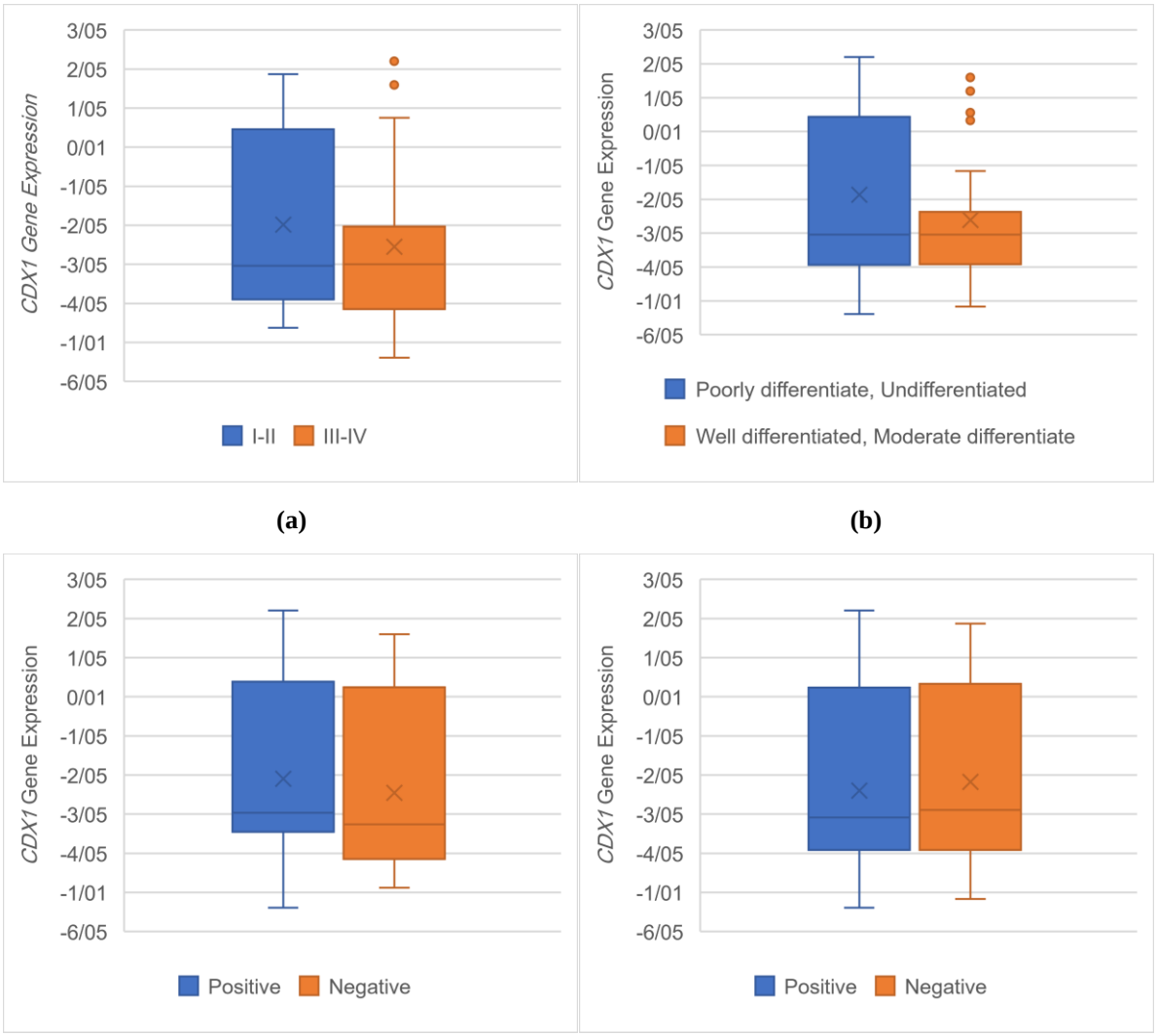
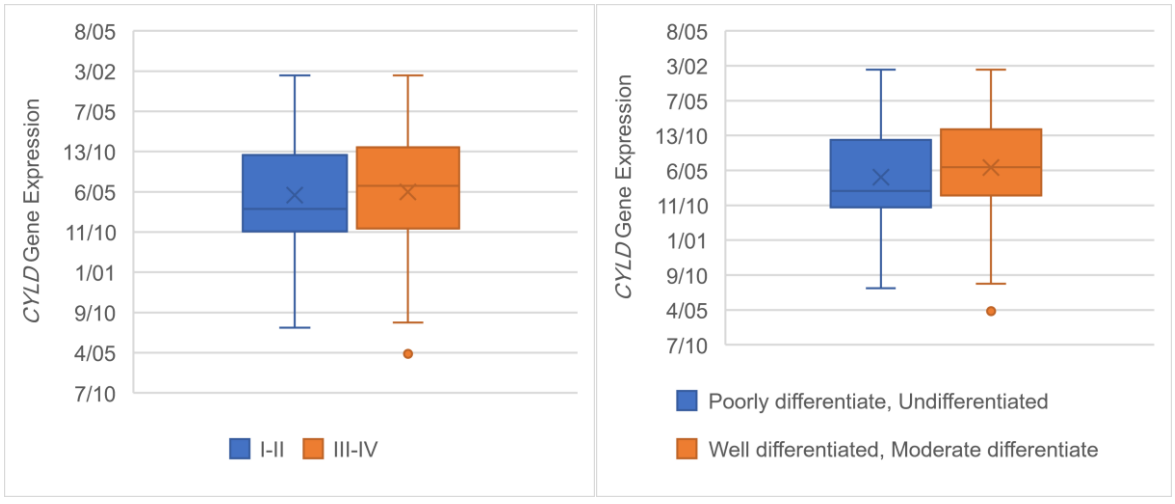
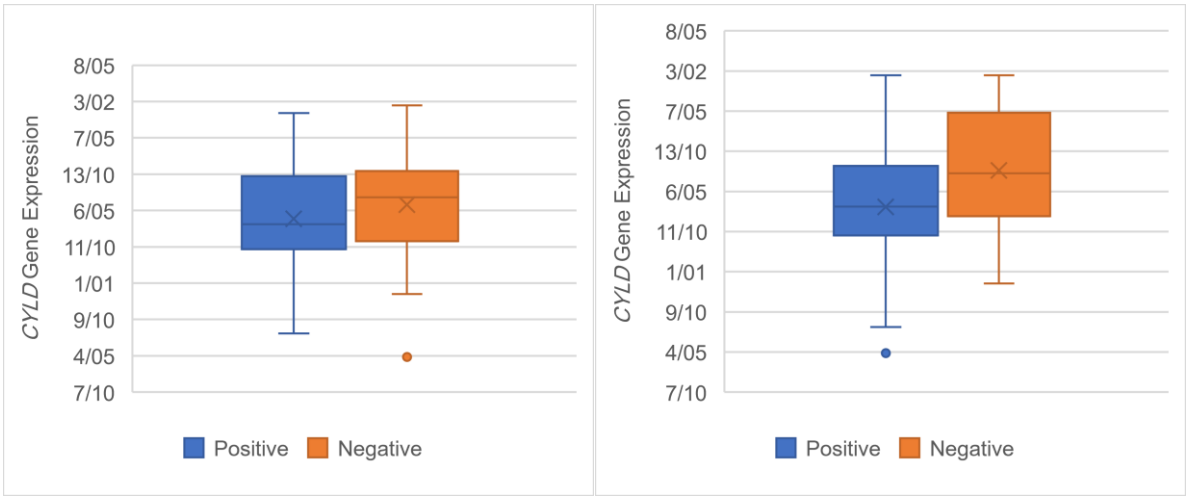


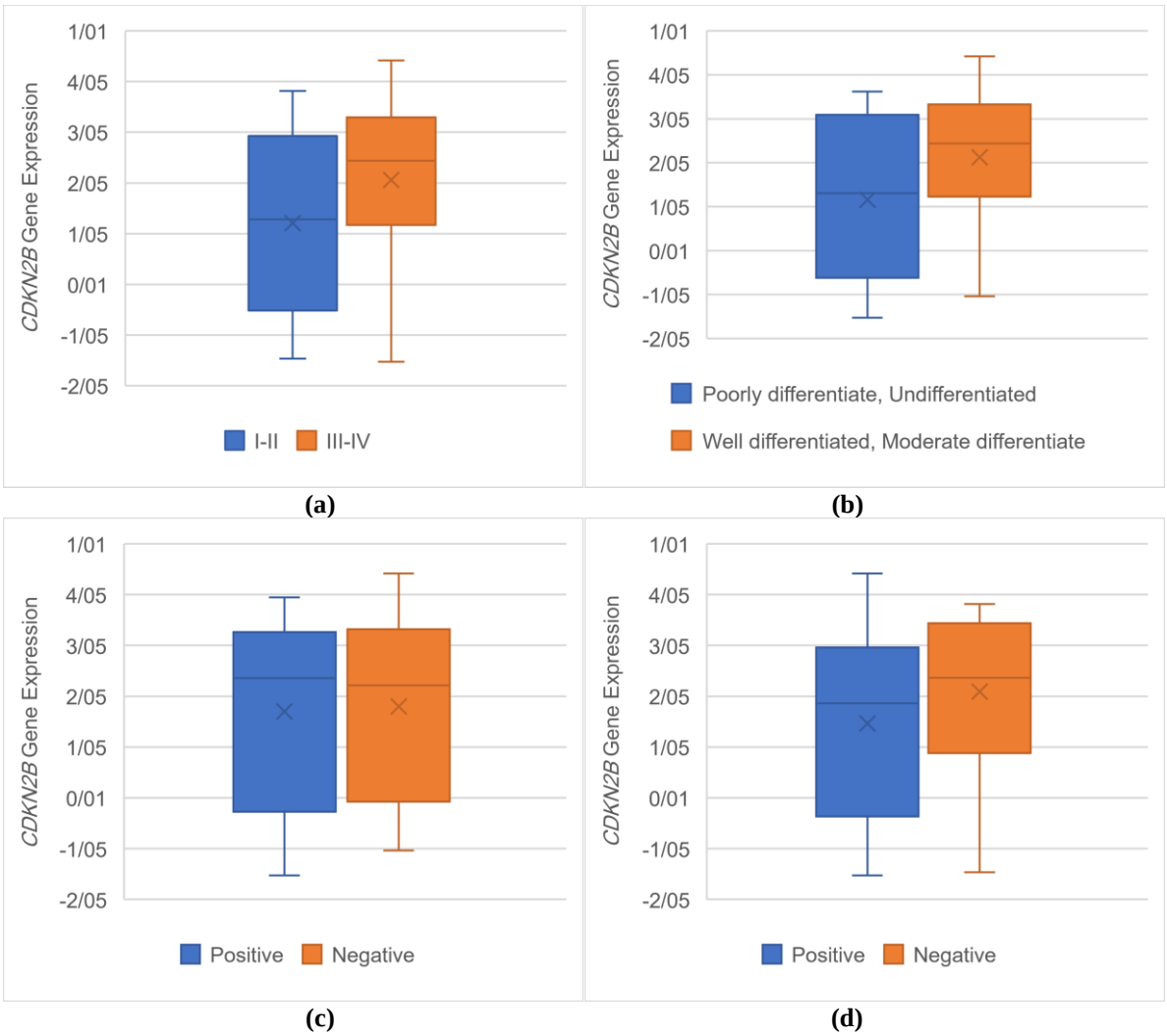
Figure 4. The Association of *CDX1* expression with clinicopathological qualifications. There was no significant association between *CDX1* downregulation with **(a)** tumor stage (P =0.7), **(b)** tumor grade (P =0.1), **(c)** lymph-node metastasis (P= 0.4) and **(d)** distance metastasis (P= 0.5).





(c) **(d)**

Figure 5. The Association of *CYLD* expression with clinicopathological qualifications. The *CYLD* expression was significantly associated with **(a)** tumor stage (P =0.03), **(b)** tumor grade (P =0.03), **(c)** lymph-node metastasis (P= 0.05) and **(d)** distance metastasis (P= 0.05)**.**



(a) **(b)**

(c) **(d)**

Figure 6. The Association of *CDKN2B* expression with clinicopathological qualifications. There was no significant association between CDKN2B upregulation with **(a)** tumor stage (P =0.5), **(b)** tumor grade (P =0.6), **(c)** lymph-node metastasis (P= 0.3) and **(d)** distance metastasis (P= 0.2).

Discussion

Transgenic expression of *CDX1* in mouse gastric epithelium causes intestinal transdifferentiation, which protects this consideration that *CDX1* is up-regulated in Barrett's metaplasia of the esophagus. Considerably, many transcriptional targets and effective activities of *CDX1* have been recognized, there remains much to learn about the mechanisms by which it encourages differentiation and, also, those by which it inhibits stemness *CDX1* action as transcription factors regulate a wide range of cellular mechanisms (6).

Additionally, *CDX1*, an intestine-specific transcription factor, is a candidate tumor suppressor gene and it manages the intestine-specific gene transcription and regulates the intestinal epithelial cell phenotype. Past investigation illustrated that the murine *CDX1* overexpression in rat normal intestinal epithelial cells regulates proliferation as a conclusion of inducing cell cycle arrest. Meaningly, this antiproliferative role may be mediated through down-regulation of the D-type cyclins (17). The *CDX1* gene is expressed in a collaborative model during intestinal progression. *CDX1* expression will last in the intestinal epithelium throughout life, notably in the crypt. The same model of *CDX1* expression was discovered in the human small intestine. Many searches have described that the *CDX1* expression is markedly down-regulated in both adenomas and carcinomas of the colon. Little is known about the molecular mechanisms that regulate the developmental and spatial patterns of the *CDX1* expression in normal intestine or what induces the down-regulation in colonic adenomas and cancers (18). Wong et al. have shown that the loss or reduction of *CDX1* is often induced by promoter methylation. Together, these observations indicate a potential role of *CDX1* loss in tumor development (19).

Recently, the expression monitoring of *CYLD* in many colorectal-related lesions and the clinical significance of *CYLD* expression in CRC have remained unclear, although, past investigation indicating that both the transcription function and the protein level of *CYLD* were downregulated in colon cancer in comparison with normal colon tissues. The difference of *CYLD* expression in the normal colorectal epithelium, benign adenoma, primary CRC and metastatic lesions was explored (20). Of particular interest, we wondered whether *CYLD* expression played a part in tumor development, progression, or metastasis and whether reduced *CYLD* expression was a good or poor prognostic factor for CRC patients. These findings strengthened the fact that *CYLD* functioned as a tumor-suppressor gene not only in the skin tumor but also in CRC. In addition, reduced *CYLD* expression was an independent factor for poor prognosis of CRC patients. Based on the evidence above, our results also recommended that the downregulation of *CYLD* might be involved in a series of important biological properties of colorectal cancer cells, such as carcinogenesis, tumor progression and metastasis (21). These findings also have implications on the tumor suppressor function of *CYLD*, as colonic inflammation in IBD patients is a risk factor for colorectal cancer. The potential association of *CYLD* gene suppression with colon cancer is more directly suggested by a study showing reduced expression of *CYLD* in colon cancer cell lines and tissue samples It is currently unknown how the *CYLD* gene is suppressed in IBD and colon cancer cells. Nevertheless, the mechanistic insight of *CYLD* gene repression has been provided by studies using other cancer models (22).

In another study, *CYLD* expression was analyzed in two of the most common human carcinomas worldwide. Colon carcinoma derives from intestinal epithelial cells and HCC derives from hepatocytes. We found reduced *CYLD* mRNA expression in all three HCC cell lines and eight colon carcinoma cell lines examined compared with normal primary cells. Additionally, reduction or loss of *CYLD* expression was found in situ in most hepatocellular and colon carcinoma compared with non-neoplastic tissue samples. Analysis on protein level confirmed these findings. Functional assays with *CYLD* transfected cell lines revealed that *CYLD* expression decreased NF-κB activity. Thus, functional relevant loss of *CYLD* expression may contribute to tumor development and progression, and may provide a new target for therapeutic strategies (11). *CDKN2B* is a cyclin-dependent kinase inhibitor and functions as a cell growth regulator that controls cell cycle G1 progression. Last investigations have acknowledged *CDKN2B* as a required tumor suppressor, and deletion of its enhancer element is related to many different malignancies. Silencing of *CDKN2B* gene expression by epigenetic modification characterize in multiple

myelomas gastric adenocarcinoma (23). Reexpression of *CDKN2B* in tumor-derived cells significantly attenuates the tumorigenic potential of the cells and delays tumor progression (24). Fluctuation of *CDKN2B*'s expression has been announced in association with many malignancies particularly, prostate, colorectal, breast, and liver cancer. Considerably, *CDKN2B* were ubiquitously expressed in colon cancer at different stages of tumorigenesis (25).

CDKN2B encoded by the INK4b-ARF-INK4a locus. It is an acknowledged tumor suppressor gene that can form a complex with *CDK4* or *CDK6* and inhibits the activation of the cyclin-dependent kinase and progression of the cell cycle. The INK4b-ARF-INK4a locus is organized by Polycomb repressive complexes. In this way, downregulation of *CDKN2B* was investigated in cancers (26). The epigenetic investigation of these genes alongside gene expression and also a mutation of other genes which are involved in GI cancers is recommended strongly.

Conclusion

It is concluded that the upregulation of *CYLD* and *CDKN2B* genes and downregulation of *CDX1* gene in tumoral tissues were impressive. Conspicuously, the modification of these genes expression can be accepted as the main biomarker in colorectal cancer.

Author contributions

RZ, PR, and FAS collected data and accomplished some sections of the study and manuscript, SMTH collected all the biopsies directly in Omid clinic and hospital by himself and also confirmed the clinical qualifications of all the patients as a gastroenterologist. ZKK controlled and confirmed the data quality, evaluated and optimized the informatics database, wrote the paper and edited it, some other essential functions containing study design, controlling the project and protocol development and also data analysis. All authors revised the article carefully, read and acknowledged the final version of the paper.

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Conflict of interests

Authors declare no conflict of interest.

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Histopathological pathogenesis of gastric adenocarcinoma in comparison with breast cancer

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Abstract

There are numerous serious varieties of cancer that are extremely difficult to treat. As a result, understanding the origins of cancer, as well as the practical application of cancer in terms of its role of diagnosis and therapy. Detecting Gastric cancer early and correctly diagnosing it histopathologically increases the odds of an effective treatment. Histopathological expertise can help speed up and simplify oncological examinations in this method. According to their various natures, breast and gastric cancers have different tissues and rates. Gastric cancer is still one of the most lethal cancers with a dismal prognosis. New gastric cancer classification based on histologic characteristics, genotypes, and molecular phenotypes aids in better understanding the peculiarities of each subtype and improves early detection, prevention, and treatment. The goal of this essay is to go over the new gastric and breast cancer classifications so that they can be used in management and therapy.

Keywords: Histopathological pathogenesis, Gastric adenocarcinoma, Breast cancer

Introduction

Breast cancer is the most common cancer in women, and unfortunately, the age of onset of this disease has decreased. The reason for this is the referral of patients in the advanced stages of the disease so that still the most common cause of death and severe disability due to breast cancer late diagnosis (1). Cancerous and non-cancerous masses in the breast are also different. Fibroadenoma is also an important tissue mass in the breast (2).The most common benign breast mass is a fibroadenoma, which is usually a painless, circular mass with a rubbery or cartilaginous consistency. Fibroadenomas are usually solitary, but in 10-25% of cases can be multiple.

The disease is more common at younger ages but can occur in all age groups 3. In most cases, these lumps are 2-3 cm at the time of diagnosis but can grow within a few months. When the fibroadenoma is larger than 5 cm, it is called Giant fibroadenoma. Definitive diagnosis of fibroadenomas is made by a combination of physical examination, ultrasound, and postoperative Fine needle aspiration (FNA) examination. On physical examination, these lumps are firm with a smooth, round wall with a rubbery consistency, there is no inflammatory reaction around them, they are fully mobile, they do not cause sagging of the back or nipple, they are often palpable, and a groove is felt when touched (3-5).

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In the classical form, a mass with definite soft and solid boundaries is seen whose craniocerebral length is less than transverse length. In the diagnosis between a cyst and a fibroadenoma, mammography cannot help much, but ultrasound clearly shows the cyst cavity. The presence of fibroadenomas does not increase the risk of breast cancer (4). Of course, neoplasms may occur in the epithelial elements of a fibroadenoma - like the epithelium of other parts of the breast - but overall cancer is very rare in a newly discovered fibroadenoma. Half of the neoplasms that occur in fibroadenomas are ICSI (in situ lobular carcinoma), 25% are infiltrative carcinomas, and 15% are intraductal carcinomas (5). The possible diagnosis of fibroadenoma in the breast must be confirmed by FNA and CNB (Core needle biopsy). Unfortunately, this stage is not well established in our country, and due to the lack of sufficient facilities for needle aspiration with ultrasound guidance and the lack of CNB in all centers, there is a strong desire to remove a fibroadenoma. Simple and digital mammography and, if necessary, magnification mammography - performed in one day for the patient. In the case of benign masses such as fibroadenoma, after a thorough and accurate history and appropriate physical examination, the patient is sent to a special doctor's room for ultrasound and simultaneous needle aspiration with ultrasound guidance (6). Ultrasound is performed with a 14 and 16 MHz probe and in addition to accurately determining the volume and nature of the mass, a new and very interesting elastography method is used to better identify the accompanying masses and the consistency of the mass. At the same time, the orthopedic surgeon solids the mass (Cystic) with the help of ultrasound, and the sample is immediately taken to the pathologist for observation. In less than an hour, the pathology and cytology results are known and the patient is sent to the CNB room to confirm the diagnosis. Core sampling is performed carefully and after examining and determining the benign nature of the mass, even in cases of large fibroadenomas, the patient is scheduled for further control and large incisions and resection of the mass are not performed (7).

The anatomical structure of the breast

Each breast is made up of lobes and lymphatics. Each breast is made up of 15 to 20 sections called lobes. Each of these lobes is made up of several smaller parts called lobes. The lobes and lobular are connected by tiny ducts called lymphatics. There are also several blood vessels and lymph vessels in each breast. Each of these lymph vessels carries a colorless fluid called lymph. All of these lymph vessels lead to small organs and beans in a shape called lymph nodes. These lymph nodes help the body fight disease and infection. Lymph nodes are present all over the body. Lymph nodes are found in groups near the breast, under the armpits, above the clavicle, and in the chest (8, 9).

Morphological and histological signs of breast cancer include the presence of a swollen or firm mass inside or near the breast or under the armpit, changes in the shape or size of the breast, the presence of wrinkles or dimples on the skin of the breast, and the release of any fluid. Apart from nipple milk, especially if this fluid is bloody, scaling, redness, or swelling on the breast, nipple, the presence of depressions in the skin of the breast, so that these depressions look like orange peel. In this case, this complication is called Peau d’orange. These symptoms may occur in some normal people and are not necessarily "specific to breast cancer" (10, 11).

Breast cancer is often very difficult to diagnose in pregnant or breastfeeding women, who usually have tender and swollen breasts. Women who are pregnant, breastfeeding, or giving birth often have sensitive and swollen breasts. In this case, the diagnosis of small tumors is very difficult and often the diagnosis of breast cancer is delayed. Because of these delays, cancer is usually diagnosed in this group of women in the advanced stages of the disease (12).

Biopsy

A tissue sample from a suspicious mass and examined by a pathologist under a microscope to look for cancer cells is called a biopsy (13).

Factors affecting the chances of recovery and treatment of breast cancer

Factors influencing recovery include the stage of cancer (mass size, axillary lymph node involvement, and distant metastasis), the status of the estrogen and progesterone receptors in the cancer cells, the status of the HER2 receptor cancer cells, the presence of general cancer symptoms, and the patient's general health (14,

15). Once breast cancer is diagnosed, tests are done to see if the cancer is present only in the breast itself or if it has spread to other organs. Some ways of spreading breast cancer include spreading through adjacent tissues, invading adjacent tissues, spreading through the lymphatic system, cancer also invading the lymphatic system and spreading through the lymph vessels to other parts of the body, and finally Through the blood, cancer invades the blood vessels and spreads through the blood to other parts of the body (16).

When cancer cells are isolated from the primary tumor and spread to other parts of the body through the blood or lymph, another (secondary) tumor forms. This process is called metastasis. The second (metastatic) tumor is the same as the first tumor. For example, if breast cancer spreads to the bones, the cancer cells in the bone are the same as the breast cancer cells. In this case, the disease is metastatic (17).

Staging of breast cancer

The stage of breast cancer represents the rate of progression of the disease in breast tissue and other organs of the body and directly indicates the survival rate of the patient following cancer. The more advanced the disease, the shorter the patient's lifespan. Breast cancer stages include Stage 0 to stage four (Stage IV) It is worth noting that the choice of treatment is based on the stage of the disease (18).

Stage 0 or intraductal carcinoma (in situ)

There are two types of breast cancer in the in-situ stage.

1. Ductal carcinoma in situ (DCIS)

At this stage of breast cancer, abnormal cells are seen non-invasively exclusively in the lining of the ducts of the breast and have not invaded the basement membrane and other parts of the breast. If the disease is not detected and treated in the DCIS stage, the cancer cells continue to grow and invade the basement membrane and other breast tissues (19).

2. Lobular Carcinoma in situ (LCIS)

At this stage of the disease, cancer cells are found only in the lips of the breast. LCIS rarely become invasive cancer, but having LCIS in one breast can increase the risk of developing cancer in the other breast (20).

Stage I

In Stage I, cancer has formed and this stage is divided into two stages: Stage IA and Stage IB.

Stage IA and Stage IB.

1. Stage IA

The tumor is less than 2cm or 2cm in size and the cancer cells have not spread outside the breast tissue.

2. Stage IB

No disease or tumor is found in the breast at this stage, and only small clusters of cancer cells (larger than 0.2 and smaller than 2 mm) are seen in the lymph nodes. Or, the tumor is 2 cm or smaller, and small clusters of cancer cells (larger than 0.2 and smaller than 2 mm) are found in the lymph nodes.

Stage II

The second stage is divided into two stages, Stage II A and Stage II B.

Stage II A

No tumors are found in the breast, but cancer is found in the axillary lymph nodes. The tumor is 2 cm or smaller in size and has spread to the axillary lymph nodes. Or the tumor size is larger than 2 cm and smaller than 5 cm and has not spread to the axillary lymph nodes.

Stage II B

The tumor is larger than 2 cm and smaller than 5 cm and has spread to the axillary lymph nodes. The tumor is larger than 5 cm but has not spread to the axillary lymph nodes.

Stage III

The third stage is divided into three stages, Stage III A, Stage III B and Stage III C.

Stage III A

No tumors are found in the breast at this stage. And only cancer is found in the axillary lymph nodes, glands that are either attached or to other parts of the breast, or cancer may even be found in the lymph nodes near the breastbone (10). The tumor is 2 cm or smaller. In this case, cancer may spread to the axillary lymph nodes,

which are either connected or to other parts of the breast, and cancer may even spread to the lymph nodes near the breastbone. The tumor is larger than 2 cm and smaller than 5 cm. In this case, cancer has spread to the axillary lymph nodes, glands that are connected or to other parts of the breast, or may even have spread to lymph nodes located near the breast bone (11). The tumor is larger than 5 cm. In this case, cancer has spread to the axillary lymph nodes, glands that are connected or to other parts of the breast, or may even have spread to the lymph nodes near the breast bone.

Stage III B

At this stage, the tumor may be of any size and cancer has spread to the chest wall or breast skin, or cancer has spread to the axillary lymph nodes, glands that are connected or to parts of the breast. It may or may not have spread to the lymph nodes near the breastbone. This stage of the disease is called inflammatory breast cancer, which is associated with skin involvement (12).

Stage III C

At this stage, there may be no signs of breast cancer or a breast tumor of any size. Or cancer may have spread to the chest wall or even to the skin of the breast. Cancer has also spread to the lymph nodes above or below the collarbone. It has spread to the axillary lymph nodes and lymph nodes near the sternum. Stage IIIC Breast cancer itself is divided into operable and non-surgical. In the operative stage, the cancer is found in 10 or more lymph nodes. The cancer is found in the lymph nodes below the clavicle. Cancer of the axillary lymph nodes is found near the sternum. Inoperable Stage IIIC cancer has spread to the supraclavicular lymph nodes (21).

Stage IV

In stage 4 breast cancer, the disease has spread to other parts of the body. These organs often include bone tissue, lungs, lungs, liver and brain (22) (Figure1).

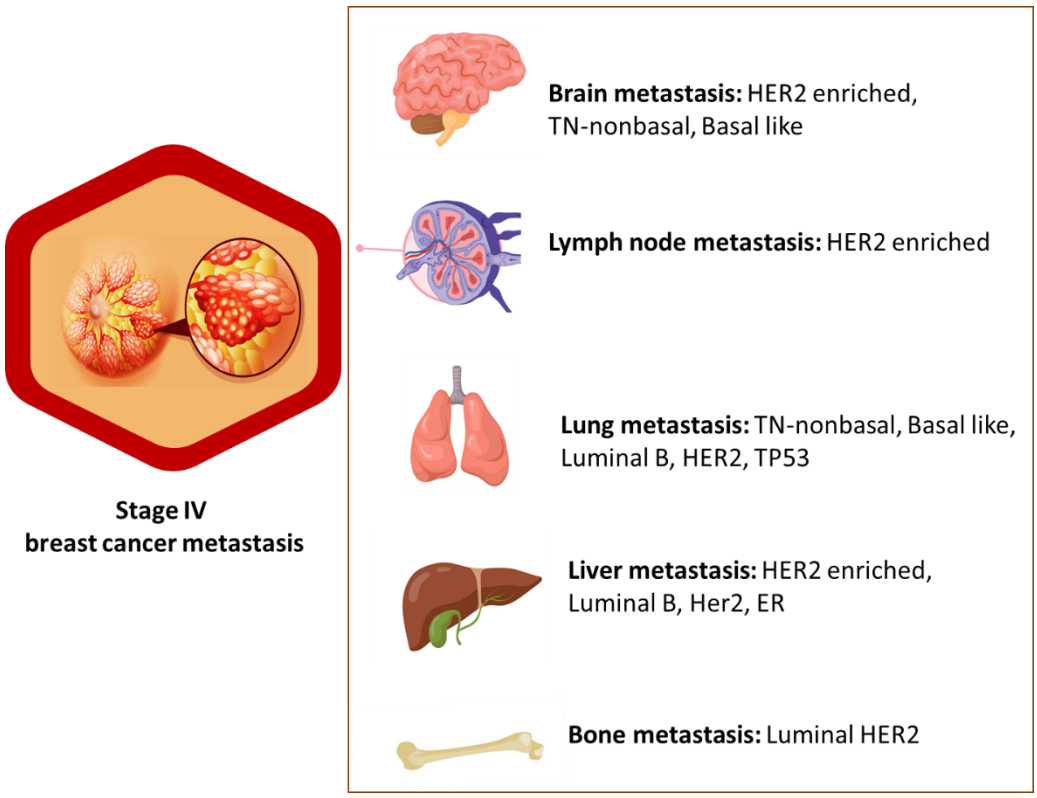

Figure 1. Advanced (metastatic) breast cancer.

Inflammatory breast cancer

At this stage, cancer invades the lymph vessels of the breast skin, causing them to become blocked and the breast to become red and swollen, and the person may feel warmth at this point. It is called d 'orange. In this case, a certain mass in the chest may not be touched due to severe swelling. This condition of breast cancer can occur in any of the stages of Stage III B, Stage III C, Stage IV (23, 24).

Recurrent breast cancer

Recurrent breast cancer is cancer that has come back after a full course of treatment. Cancer may come back in the breast tissue, chest, or any part of the body (25).

Gastric cancer

Gastric cancer (GC) is a type of cancer and its most common form is adenocarcinoma or glandular cancer of the stomach (26).
Other less common types of stomach cancer include lymphoma (cancer of the lymphatic system) and sarcoma (cancer of connective tissue such as muscle, fat, or blood vessels). Gastric cancer kills about one million people worldwide each year (27).
It kills many people around the world and is twice as common in men as women and is the fourth most common cancer in the world. It is more common in people with blood type A. Embryonic cell debris in the esophagus and upper third of the stomach is a risk factor for gastric cancer. Embryonic cell debris has the potential to become cancerous with routine diagnostic tests, X-rays and CT scans that are not detectable (26, 28).
Gastric cancer, also known as abdominal cancer, is cancer that occurs in the stomach and upper abdomen. The prevalence of gastric cancer is relatively low in the United States, and it is more common in countries such as China and Japan. Gastric cancer is divided into several types, and the most common type (about 90 to 95% of all types) is cancer that occurs in the glandular area of the stomach. Gastric cancer may be cured if diagnosed early, but unfortunately in the advanced stages of the disease the result is not very satisfactory (29, 30). It should be noted that the presence of risk factors does not always mean getting the disease and only increases the conditions for getting the disease.
Helicobacter pylori infection leading to chronic gastritis. Of course, many people who carry this germ will never get stomach cancer (31).

Helicobacter pylori (H. pylori)

Helicobacter pylori is a curved gram-negative bacillus that lives in the labia of the gastric mucosa and sometimes in the duodenum and esophagus. This bacterium is by no means part of the natural flora but causes chronic superficial and diffuse inflammation in infected people in the stomach (32).

The biochemical properties of this bacterium are the production of the enzyme urease. The disease is usually transmitted through oral feces. This is because the bacterium is more common in people who are in poor health or in people who live in groups. The prevalence of Helicobacter pylori is primarily dependent on age and geographical area and its prevalence are the same in men and women (33).

Helicobacter pylori can survive in the gastric mucosa, where many bacteria are unable to survive. But when the acidity of the stomach decreases, other bacteria can survive because it may compete with other bacteria. Diagnosis is based on radiology, endoscopy, urease test, and medical history. But these methods are time-consuming and sometimes aggressive, and their sensitivity is not entirely clear. However, the ELISA method has eliminated this problem by identifying specific antibodies so that the desired results can be achieved in a short time with a non-invasive method (34).

Various studies have shown the presence of antibodies against Helicobacter pylori and gastric disease. There is a clear relationship between serum IgM antibody levels and clinical tissues so that an increase in serum immunoglobulin levels is seen in acute gastritis. This test is very useful as a rapid screening test and also in the early detection of Helicobacter pylori infection because the immune response often occurs before clinical signs (Figure 2) (35, 36).

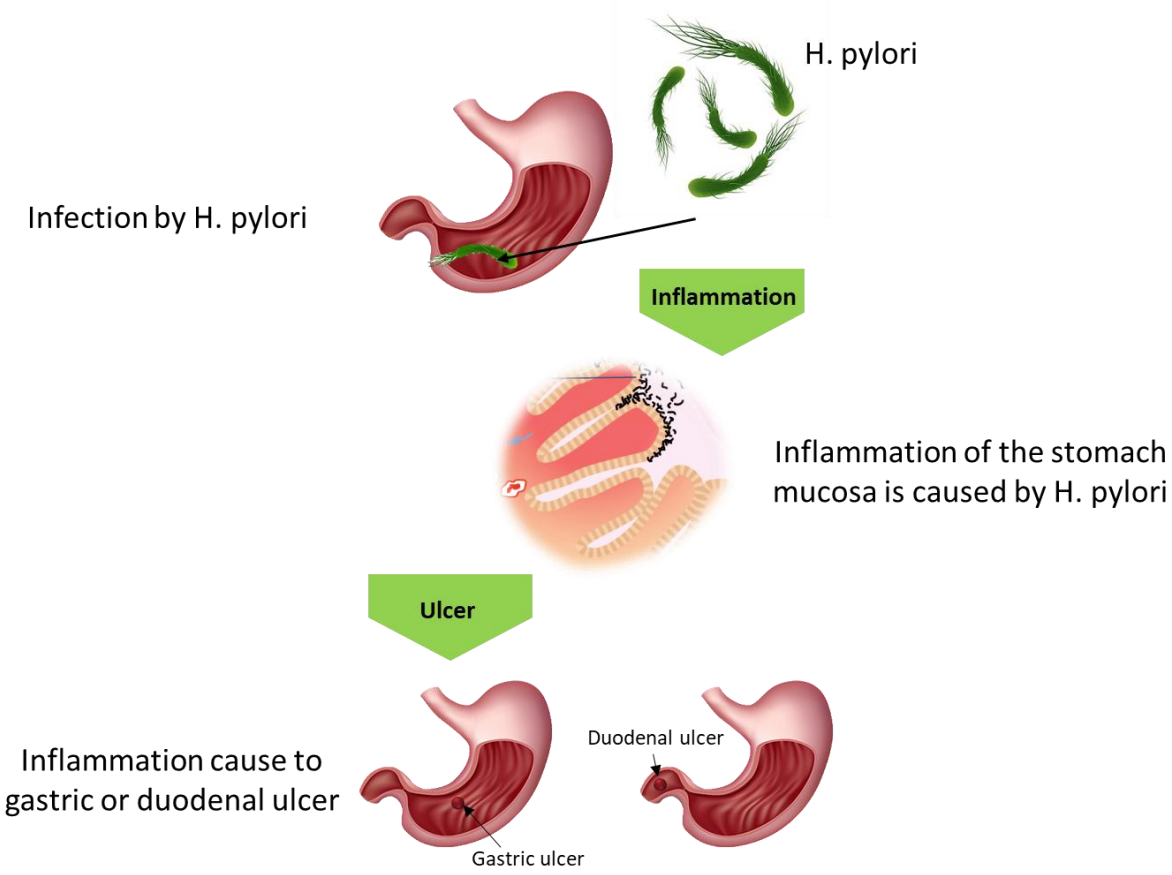

Figure 2. The process of gastric cancer by Helicobacter pylori.

Conclusion

Globally, GC is the second leading cause of mortality from cancer. Clinical behavior, the biology of tumor and outcome may all be predicted by histologic categorization. The disease is split into two forms, diffuse and intestinal, based on the current Lauren classification, with the latter having a better prognostic. Diffuse-type GC is the most common subtype in the general population, and it is linked to a poorer prognosis. BC is the most frequent cancer in the world, with a high mortality rate, particularly among women. Early detection and proper medical care might raise the chances of survival. Because the diagnosis procedure is time-consuming and the results may differ amongst pathologists, the computer-Assisted Diagnosis (CAD) system is critical for enhancing precision.

Author contribution

SAN accomplished the data processing, investigated, wrote the whole manuscript, revised and managed the manuscript.

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Conflict of interest

There are no conflicts of interest.

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The burden of care and its correlates in family caregivers of breast cancer patients undergoing chemotherapy in Sari, Iran, in 2020

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Abstract

Introduction: Caring for patients with cancer can often give rise to numerous physical and mental health problems or even exacerbate them among family caregivers. The present study aimed to reflect on the burden of care and its correlates affecting family caregivers of breast cancer (BC) patients undergoing chemotherapy.

Materials and Methods: This descriptive-analytical cross-sectional study of correlational type was conducted on a total number of 163 family caregivers of patients with BC undergoing chemotherapy at Imam Khomeini Teaching Hospital and Baghban Specialized Center based in the city of Sari, Iran, in 2020. For this purpose, the study samples were selected through the available sampling technique. As well, the demographic characteristics information form and the Caregiver Burden Inventory (CBI) were employed for data collection. The data were ultimately analyzed using the SPSS Statistics software (ver. 21).

Results: The study results revealed that 72 family caregivers (44.17%) were experiencing high levels of burden of care (>36). Moreover, caregivers' level of income, number of chemotherapy sessions, duration of disease, and patients' insurance coverage status were significantly correlated with caregiver burden, which were evaluated in the multiple logistic regression analysis. This model could further explain 25.2% of variance in the burden of care severity in these informal caregivers ($p<0.001$, Chi-square [$\chi 2$] statistic=33.9). The multiple logistic regression analysis was also utilized to determine the effect of the most important dimensions of the burden of care and this model could account for 94.8% of variance in the burden of care severity among these family caregivers, wherein developmental, social, physical, and time dependence dimensions respectively had the greatest impacts on compounding caregiver burden.

Conclusion: The study results indicated high levels of burden of care in family caregivers of patients suffering from BC. Therefore, it was recommended to consider the burden of care correlates and to plan for proper interventions, according to the findings in the present study, in further research.

Keywords: Burden of Care, Breast Cancer, Demographic Characteristics, Patient Companion

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Introduction

Cancer is known as one of the most important diseases in the 21st century (1), so a significant share of health care programs has been allocated to this condition (2). Asia also has the highest prevalence rate of cancer (48% of the cases over the world) among all continents. In this respect, 1 out of every 8-10 women across the world and 1 out of every 10-15 women in Iran, have a higher chance of developing breast cancer (BC) (3). As well, this type of cancer in Iran accounts for 21.4% of all reported cases (4). The incidence of this condition in this country, particularly in central and Northern provinces is considerably on the increase (5). The onset age of developing BC in Iran is also one decade lower than that in Western countries (6). This disease can thus affect various aspects of life in women. Diagnostic tests and medical procedures, complications induced by treatments such as nutritional problems, fatigue, nausea, vomiting, and pain, as well as disruptions of social relationships and care/treatment costs are among other consequences of this condition (7). Given the debilitating nature of cancer and its related care/treatment, most of those suffering from this disease need more support by informal caregivers (1), who might have a sense of despair, loneliness, and failure in assuming their own duties due to no education with regard to the disease and receiving no information support in this domain (8). As 55% of care is provided by informal caregivers, such people mostly fail to meet their own needs in daily living and even ignore them to provide care for their loved ones (9). The mean time of care for cancer patients has been estimated by 8.8 hours per day (10) and family caregivers are required to fulfill not only their daily routine activities but also their obligations in the workplace (11). Under such conditions, if family caregivers fail to manage the time of care for patients and that allocated to their own personal issues, they will progress towards the burden of care (10). Here, caregiver burden is a general term to describe physical, emotional, and socioeconomic costs of care. It has been also defined as the product of the imbalance between costs of patient care and those for care facilities (12). Although the caring role in caregivers can raise a sense of affection and love in these individuals and bring some benefits such as a meaningful life, more intimacy in family relationships, respect for oneself and others, as well as a sense of satisfaction (13), it might be accompanied by problems such as burnout, anxiety, depression, insomnia, decreased appetite, and hypertension (14, 15). Studies have further shown that factors such as duration of care, family economic status, social support, number of caregivers, type of disease, increased duration of disease, and clinical symptoms in patients can have significant effects on the burden of care (16, 17). Given the high importance of caregivers in support of patients to deal with BC and considering the growing population with this condition and little research on the burden of care, this study aimed to evaluate the burden of care and its correlates in family caregivers of patients with BC undergoing chemotherapy.

Materials and Methods

This descriptive-analytical cross-sectional study of correlational type was conducted in the chemotherapy wards of Imam Khomeini Teaching Hospital and Baghban Specialized Center based in the city of Sari, Iran, in 2020. The statistical population included all caregivers of BC patients referred to these centers, of which 163 caregivers of the patients undergoing chemotherapy were selected.

The sample size was further estimated to be 163 individuals based on the study by Adili and Dehghan Araie (18), using the following calculation formula:

$$n = \frac{(1.96)^2 \delta^2}{d^2} = \frac{(1.96)^2 (26)^2}{4^2} = 163$$

Of note, this research project was approved by the Research Ethics Committee of Mazandaran University of Medical Sciences, Sari, Iran (IR.MAZUMS.REC.1399.6442). During 11 months (from April to January 2020), the convenience sampling technique was performed after providing explanations and obtaining written consent from the family caregivers. Ethical considerations in this study were also met by observing the confidentiality of information, voluntary participation in the research, possibility of withdrawing from the project without time restrictions, and explaining the objectives and the research procedure to the study samples.

The primary family caregivers of the patients included spouse, sibling, and parent. As well, the inclusion

criteria were literacy (i.e., reading and writing ability), age over 18 years, no mental illnesses, and approvals that these individuals were the primary caregivers and companions. Moreover, those receiving counseling and psychology services, taking sedatives and tranquilizers, or caring for patients with underlying diseases or the ones with metastatic progression were excluded.

The data in this study were collected using two-part questionnaire. The first part contained the demographic characteristics information form about the family caregivers (including gender, age, marital status, level of education, occupation, and kinship) and the patients (such as gender, age, marital status, level of education, occupation, housing, level of income, duration of disease, insurance coverage status, and number of chemotherapy sessions).

The second part of the questionnaire was the 24-item Caregiver Burden Inventory (CBI), developed by Novak and Guest (1989), which was utilized to evaluate the level of burden of care perceived by the caregivers in an objective and subjective manner. This questionnaire measured the burden of care in five dimensions, i.e., time dependence, developmental, physical, social, and emotional (18). The total score could be also from zero to 96, in which scores of <35 indicated lower level of burden of care and the scores of ≥36 represented higher levels of burden of care. The participants could further determine the degree of their experiences with each situation in a five-point Likert-type scale - completely false (0 point) false (1 point), to some extent (2 points), true (3 points), and completely true (4 points).

The CBI was translated by Abbasi et al. into Persian and its validity was further confirmed through content validity method. In a study on patients with end-stage cancer, the Cronbach's alpha coefficient had been similarly calculated by 0.90 (2). In order to analyze the data, they were firstly summarized using descriptive statistics including mean and standard deviation (SD) for the quantitative data and frequency tables for the qualitative ones. Then, descriptive statistics, independent-samples t-test, Chi-square test, and Spearman's rank-order correlation were employed to examine the relationships. The final analysis of the relationships was done through the multiple logistic regression analysis at a significance level of 0.05, using the SPSS Statistics software (Ver. 24). After the normality of data was examined, the Kolmogorov-Smirnov test was used for inferential analysis.

Results

A total number of 163 primary family caregivers, most of them as the spouse of 85 patients (52.1%), with a mean age of 43.6±11.7 years were studied. The results also revealed that 55.83% and 44.17% of the family caregivers had low and high levels of burden of care, respectively. Given the demographic characteristics information, most of the family caregivers in this study were male caregivers (n=97, 59.5%), married individuals (n=136, 83.4%), those holding university degrees (n=66, 40.5%), homemakers (n=45, 27.6%), the ones owning a house (n=132, 81%) and cases with a moderate-to-high level of income (n=129, 79.2%). According to the demographic characteristics of the patients, majority of them in this study were married (n=141, 86.5%), had high school diploma and lower degrees (n=118, 72.4%), owned a house (n=128, 78.5%), and a moderate level of income (n=77, 47.2%) as well as insurance coverage (n=150, 92%). Of these patients, 149 cases (91.4%) also had chemotherapy-induced complications (Table 1). The mean age of the patients in this study was 46.7±10.6 years old, the mean length of chemotherapy was 6.26±4.3 sessions, and the mean duration of disease from diagnosis was 7.86±6.7 months.

The logistic regression analysis was further used to determine the correlates affecting the burden of care in family caregivers. First, the univariate mode of the logistic regression analysis was implemented and the variables whose values were <0.3 were selected for the multiple logistic regression model. Next, employing the multivariate logistic regression analysis and the backward stepwise selection method, the given variables were considered for the model with the probability of excluding each variable by 0.1. The variables of the family caregivers' level of education, occupation, level of income, and kinship along with the patients' marital status, occupation, level of income, number of chemotherapy sessions, chemotherapy-induced complications, duration of disease, and insurance coverage status whose univariate p-values were <0.3 were also taken into consideration for the final model of the multiple logistic regression analysis. Within the final model, the four variables of family

caregivers' level of income as well as number of chemotherapy sessions, duration of disease, and patients' insurance coverage status remained.

Table 1. Demographic characteristics of family caregivers along with demographic/clinical characteristics of BC patients.

Groups	Variables	Categories	Descriptive indices	
			Frequency	Percentage
Family caregivers	Gender	Male	97	59.5
		Female	66	40.5
	Marital status	Single	22	13.5
		Married	136	83.4
		Divorced	5	3.1
	Level of education	Lower than high school diploma	46	28.2
		High school diploma	51	31.3
		University degree	66	40.5
	Occupation	Unemployed	9	5.5
		Employed	43	26.4
		Worker	7	4.3
		Farmer	13	8
		Self-employed	37	22.7
		Retired	9	5.5
		Homemaker	45	27.6
	Housing	Owner	132	81
		Tenant	31	19
	Level of income	Low	34	20.9
		Moderate	103	63.2
		Good	26	16
	Kinship	Spouse	85	52.1
		Child	37	22.7
		Brother or sister (sibling)	22	13.5
		Father or mother (parent)	8	4.9
		Others	11	6.7
Patients	Marital status	Single	5	3.1
		Married	141	86.5
		Divorced	8	4.9
		Widowed	9	5.5
	Level of education	Illiterate	10	6.1
		Lower than high school diploma	45	27.6
		High school diploma	63	38.7
		University degree	45	27.6
	Housing	Owner	128	78.5
		Tenant	35	21.5
	Level of income	Low	54	33.1
		Moderate	77	47.2
		Good	32	19.6
	Chemotherapy-induced complications	Yes	149	91.4
		No	14	8.6
	Insurance coverage status	Yes	150	92
		No	13	8

The regression analysis was similarly performed to determine the effect of family caregivers' level of income on chemotherapy sessions, duration of disease, and insurance coverage status of the patients and the burden of care in family caregivers (Table 2). The logistic regression model was also statistically significant ($p<0.001$, Chi-square [$\chi 2$] statistic=33.9). This model could determine 25.2% of the variance of the burden of care severity on family caregivers and correctly explained 67.5% of the effects of the burden

of care on these individuals. The study results revealed that the family caregivers with higher levels of income had experienced lower levels of burden of care in different aspects of life. For example, in family caregivers with good levels of income, the burden of care for their patients had reduced by 1.194 times compared with low-income cases. Each additional chemotherapy session had correspondingly resulted in higher levels of burden of care for the BC patients as much as 1.229 times ($p<0.001$). Moreover, the results of this study showed that an increase in the duration of disease from the time of diagnosis (namely, months) had decreased the family caregivers' burden of care in different aspects of life ($p=0.005$). In addition, in patients who benefitted insurance coverage, the caregivers' level of burden of care had significantly reduced ($p<0.001$).

Table 2. Logistic regression analysis results to investigate the relationship between burden of care and its correlates.

Variables	Beta coefficient	Standard error (SE)	Wald statistic	Degree of freedom (DF)	P-value	Exponentiation of the B coefficient (Exp [B])	95% confidence interval (CI) for EXP (B)	
							Lower bound	Upper bound
Y-intercept	-2.12	0.697	9.255	1	0.002	0.12	-	-
Family caregivers' level of income (low)	-	-	11.81	2	0.003	-	-	-
Family caregivers' level of income (moderate)	-1.177	0.457	6.63	1	0.01	0.308	0.126	0.755
Family caregivers' level of income (high)	-1.61	0.586	7.85	1	0.005	1.194	0.062	0.611
Patients' number of chemotherapy sessions	0.207	0.059	12.17	1	<0.001	1.229	1.095	1.38
Duration of disease	-0.108	0.039	7.75	1	0.005	0.898	0.832	0.969
Insurance coverage status (No)	2.188	0.883	6.145	1	0.013	8.921		
Insurance coverage status (Yes)	-3.214	0.892	12.968	1	<0.001	0.04	1.581	50.33

The logistic regression analysis was also employed to identify the most important dimensions of the burden of care among family caregivers. Accordingly, time dependence, developmental, physical, social, and emotional dimensions were considered. Initially, the univariate model of the logistic regression analysis was implemented and the variables whose p-values were <0.3 were selected for the multiple logistic regression model. Afterward, using the multivariate logistic regression analysis and the backward stepwise selection method, the given variables were considered for the model with the probability of excluding each variable by 0.1. All the given dimensions whose univariate p-values were <0.3 were taken into account in the final model of the multiple logistic regression analysis. In the final model, time dependence, developmental, physical, and social dimensions remained.

The regression analysis was also performed to determine the effect of time dependence, developmental, physical, and social dimensions on the burden of care in family caregivers (Table 3). The logistic regression model was also statistically significant ($p<0.001$, $\chi 2$ statistic=200.4). This model could account for 94.8% of the variance of the burden of care severity on the family caregivers and correctly explained 96.3% of the effects of the burden of care on caregivers. The study results revealed that the increase in each unit in the values of time dependence ($p<0.021$), developmental ($p<0.006$), physical ($p=0.005$), and social ($p=0.005$) dimensions led to a growth in the caregivers' burden of care by 1.71, 3.8, 2.2, and 3.39, respectively.

Table 3. Results of logistic regression analysis to investigate the relationship between burden of care and its dimensions.

Variables	Beta coefficient	SE	Wald statistic	DF	P-value	Exp (B)	95% CI for EXP (B)	
							Lower bound	Upper bound
Y-intercept	-25.16	8.25	9.29	1	0.002	000	-	-
Time dependence	0.534	0.231	5.35	1	0.021	1.71	1.085	2.68
Developmental burden of care	1.33	0.486	7.54	1	0.006	3.79	0.465	9.84
Physical burden of care	0.79	0.28	7.86	1	0.005	2.197	0.268	3.81
Social burden of care	1.22	0.43	8.04	1	0.005	3..39	1.458	7..89

Discussion

This study aimed to evaluate the burden of care and its correlates in family caregivers of BC patients undergoing chemotherapy. The results showed that almost half of these informal caregivers (44.17%) had experienced high levels of burden of care (>36), which was lower compared with the findings in Salmani et al. (20) who had reported the burden of care in Iranian caregivers of patients admitted to the oncology ward by 81% and in the survey by Gabriel (21) in which the burden of care in the primary caregivers of the patients with BC in Nigeria had been 86.7%. It seems that the metastasis and the underlying conditions could be the main reasons for this discrepancy. It should be noted that the patients in the present study were undergoing chemotherapy, the exclusion criteria were metastatic BC and underlying diseases. In the survey on the patients with advanced cancer, living in Northern England, Higginson (22) had also found that the patient caregivers were experiencing a lower level of burden of care (18.5±11). The researchers had attributed such findings to receiving domestic support services to the caregivers (23). In any case, cancer might put too much mental strain on family members, especially patients' spouses, and the evidence shows that they suffer from the highest levels of burden of care (24) because they are closer to their own patients with regard to emotional and physiological problems and feel more responsible in caring for their patients, which can produce greater levels of burden of care among them.

In the present study, the higher the caregivers' level of income, the lower the level of burden of care, which was consistent with the reported results by Gabriel (21) and Vahidi (25), examining the burden of care among the caregivers of BC patients. They also highlighted the relationship between the low level of income and the increased level of burden of care. Some studies had similarly demonstrated a significant correlation between economic status and low level of income and

higher level of burden of care (26- 28). These findings were additionally observed in the research by Hanratty, wherein most caregivers had to quit their jobs to provide full-time care to their patients, leading to further financial problems as well as elevated level of burden of care (29). As a result, more support for low-income caregivers to reduce the levels of burden of care seems necessary.

Besides, this study showed that a rising trend in the number of chemotherapy sessions could produce the burden of care. Given that weakness and disability increase in patients during chemotherapy sessions and the patients might be subjected to new complications at the end of each session, this issue leads to higher level of burden of care among caregivers.

The results of this study suggested that the burden of care reduced as the disease prolonged from diagnosis to treatment. In conflict with the present study, Germain et al. researched the burden of care in the elderly patients with cancer (30). The age of the patients in both studies could be the reason for the different results because older adults might demand more care. On the other hand, the patients in the present study were young or middle-aged, so they could gradually perform many diagnostic tests before treatment on their own, and they needed no constant caregivers.

The study results indicated that the caregivers whose patients were benefiting more support from insurance services had lower levels of burden of care, which was in line with the survey by Hu and Peng (31) on the burden of care in caregivers of patients with lung cancer and the findings by Johnson (32) on the burden of care among caregivers of oncology patients receiving chemotherapy. In these studies, the insurance coverage status and social support had been reported as the factors affecting the burden of care in the caregivers. It seems that the role of authorities committing insurance caregivers to provide medications for these patients has a significant impact on minimizing the level of burden of care. The results of the present study were not in agreement with the reports by Adili (18), exploring the relationship between the burden of care and the patients' quality of life in caregivers of BC patients. The reason for this discrepancy could be in the time of diagnosis, which was only three months in the survey by Adili, when it seems that the financial resources in family could still meet the financial needs of the patients. In the present study, however, this time was longer.

Since family caregivers must meet their own needs and those of patients, they suffer from additional levels of burden of care in all physical, emotional, and socioeconomic aspects along with disruption in their caring roles (33). This study showed that the most frequent burden of care among the primary caregivers was related to developmental, social, physical, and time dependence dimensions, which was in line with the survey by Ghane et al. (34), reporting the developmental burden of care as the most common dimension in the family caregivers of patients undergoing hemodialysis. However, Abbasi et al. (35) had mentioned the most frequent burden associated with the emotional dimension of care for patients receiving hemodialysis. In a study on colorectal cancer (CRC) patients, Bakim (36) had revealed that aging could lead to higher levels of burden of care in the caregivers. Since the increase in age in the caregivers reduces the emotional dimension of the burden of care following gaining experience (37) and a rise in physical, developmental, and time dependence dimensions (35), this can be justified with regard to the mean age of the caregivers (46.7±10.6 years old). Unsar et al. (38) had also indicated that the caregivers of cancer patients had to care for them all day long, so they had no spare time for themselves. Employment outside the home could similarly create an elevated level of burden of care due to the conflict between work and caring responsibilities (28). As well, Shafie Zadeh et al. (39), investigating the relationship between the burden of care and the demographic characteristics of the caregivers of the elderly with Alzheimer's disease, had found that these caregivers had experienced the maximum level of burden of care related to the time dependence dimension. It seems that the duration of care for such patients, depending on the type of the disease, can be a factor affecting the burden of care severity as the Alzheimer's disease needs constant care, while in this study, the patients did not demand full-time care according to the caregivers.

At the end of the questionnaire and according to the survey conducted among the caregivers to offer their suggestions with regard to the burden of care, financial and insurance support especially for the provision of medications and information on how to take care and deal with the complications in the patients were among

the issues noted by most of the caregivers. Studies have further shown an inverse relationship between the burden of care and social support (40, 41). In other words, caregivers may have more time and energy to care for oneself and to meet their own needs whenever they take advantage of higher levels of social support from family and professional institutions.

It seems that the use of different sampling techniques, variations in receiving care services, support services, cultural discrepancies, types of organizations involved in cancer care/treatment, as well as the most recent breakthroughs in BC care/treatment can be among the reasons for inconsistencies in the results of such studies.

Since this study was conducted during the coronavirus disease 2019 (COVID-19) pandemic and some caregivers were reluctant to complete the questionnaires manually, a number of the questionnaires were unavoidably done through interviews, which could increase the possibility of unreal responses by them. It was also possible that the caregivers tended not to talk about the burden of care or underestimate it, and even feel guilty to tell the truth in this regard.

Conclusion

The study results revealed that the caregivers of the BC patients were suffering from the burden of care during chemotherapy. Based on the study findings, caregivers' level of income, number of chemotherapy sessions, duration of disease, and insurance coverage status are significantly correlated with their burden of care. The correlation between some demographic characteristics information and the burden of care among the caregivers of this category of patients is further highlighted. Accordingly, information is provided in order to improve the existing situation of the caregivers and to reduce the burden of care. If the caregivers' burden of care is mitigated, they can play their caring roles better. Given the results of the present study, there is a need to reflect more on the caregivers' developmental and social dimensions of the burden of care. It is also recommended to provide nurses with the outcomes of the most recent studies on caring for cancer patients in the form of training courses to help them meet the educational needs of informal caregivers in order not to feel frustrated and isolated. Caregivers must be also supported by health care providers and a person should be always available as a supporter to respond to the questions posed by these caregivers and help them have lower burden of care. Health care team members should remember that the main respondents to cancer patients are their primary caregivers, whose value should never be overlooked in the health care team. Technology resources including telephone follow-up, virtual care guide, and online support groups can be thus effective in these conditions.

Author contributions

MA, MBN, JYCh and MA wrote and completed the article. SRB designed and edited the manuscript. All authors confirmed the final edited version.

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Conflicts of interest

There are no conflicts of interest.

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Investigation of self-harm cases and related factors

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Abstract

Introduction: Self-harm is a deliberate and non-lethal act in which a person intentionally injures himself or consumes a substance in excess of the prescribed amount. Self-harm injuries are also seen in forensic clients. Self-mutilation in these people is done in order to achieve a goal and is accompanied by claims of delusion of others. The importance of this issue is the necessary urgency in differentiating it from suicide, malice, mock disease and conversion diseases. In this study, we have examined self-harm and some related factors.

Materials and Methods: In a retrospective cross-sectional descriptive study, a checklist including two sections of demographic information and self-harm information was prepared to collect the necessary information based on a review of the texts and was completed by the researcher for each case. The obtained data were analyzed using descriptive tests of SPSS statistical software.

Results: Out of 783 cases, 467 (59.6%) were men and the mean age of 783 patients was 28.25 ± 8.38 years. A higher percentage of clients had more lesions on the left side of their body (61.4%), referred with a scratch lesion (46.0%), referred to a lesion in the arm area (25.7%) and of hard-edged objects used for self-harm.

Conclusion: In the face of someone who self-harms, two things should be considered, one is to achieve an appropriate treatment path considering the possibility of a mental illness that is more relevant to hospital physicians and the second is a legal view of this. From the perspective of forensic science to differentiate the damage caused by conflict and self-harm.

Keywords: Self-harm, Forensics, Aggression

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Introduction

Aggression is defined as behavior that aims to harm oneself or another. Aggression in humans represents the instinct of death as opposed to the instinct of life in action. This instinct destroys others if it can, and if it fails to target others aggressively, it will turn to itself and manifest itself as self-harm and suicide (1). Compared to suicide, self-harm is done to change current emotions, but suicide is done to end current emotions. People who self-harm are usually considered unpopular and problematic, difficult to tolerate, and many of whom have severe social and personal problems and need care (2). Self-harm is a deliberate and non-lethal act in which a person intentionally injures himself or herself in various forms such as poisoning (drugs, alcohol, etc.), interfering with wound healing, jumping from a height, interfering with wound healing, or shooting himself (3). Self-harmers cite a wide range of causes for self-harm, including energy gain, addiction, body control, sexual pleasure, and uniqueness (4).

Self-harm injuries are also seen in forensic clients. Self-mutilation in these people is done to achieve a goal and is accompanied by claims of delusion of others. On the other hand, motivations in self-harming people were classified into 4 groups, which were in the form of involuntary negative reinforcement (stopping bad feelings), involuntary positive reinforcement (self-punishment), negative social reinforcement (not going to work) and positive social reinforcement (Draw attention) (5). The prevalence of self-harm is higher in early adulthood and can be the first manifestation of a mental illness. It is also more common in people with a history of child sexual abuse (6). Alcohol and substance abuse are more common in these people, and a higher percentage of these people have long-term mental health problems. The younger the age of onset of self-harm, the more unfavorable the prognosis and the longer and longer periods and the variety of self-harm methods (7). Today, with the advancement of genetics, theories have been proposed to indicate the relationship between specific genetic patterns and psychiatric disorders (8). In this study, we have examined self-harm and some related factors.

Materials and Methods

In a retrospective cross-sectional descriptive study, 783 patients were examined for self-harm. To collect the necessary information based on the review of the texts, the checklist includes two sections of demographic information (age, sex, marital status, occupation, education, history of alcohol and smoking, history of suicide and previous self-mutilation, presence of tattoos) and information related to Self-harm (location of injury, side of injury, number of injuries, impact device and number of lesions) was prepared and completed by the researcher for each case. The data were analyzed using descriptive tests of SPSS statistical software. Continuous variables were presented as mean ± SD and discrete data as frequency percentage.

Results

Out of 783 clients due to self-harm, 467 (59.6%) were men and the mean age of 783 patients was 28.25 ± 8.38 years, while the youngest was 16 years old and the oldest was 60 years old.

Most forensic clients who had self-harmed were unemployed (45.5%), with a diploma-postgraduate degree (37.2%), married (50.6%), right-handed, with more lesions on the left side of their body. (61.4%), no previous history of self-harm (65.4%), no history of mental illness (76.4%), no history of suicide (0.93%), no tattoos on body surfaces (81.5%) 3), no alcohol consumption (75.7%), no smoking (65.3%). The frequency of scratch lesions (46%) was higher than bruises, abrasions, scratches, cuts, tears and burns. The highest frequency distribution of lesions with lesions was in the arm area (25.7%), followed by lesions in the forearm area (19.9%) and then lesions in the neck area (15.3%) (Table 1). A higher percentage of clients presented with a lesion (64.7%) (Figure 1).

Table 1. Frequency distribution of the lesions area created by self-harming individuals.

The location of the lesion	face	Neck	Arm	Forearm	Front of the body	Back of the body	Thigh	Leg	Total
Number	35	120	201	156	125	70	41	35	783
Percent	4.5	15.3	25.7	19.9	16.	8.9	5.2	4.5	100

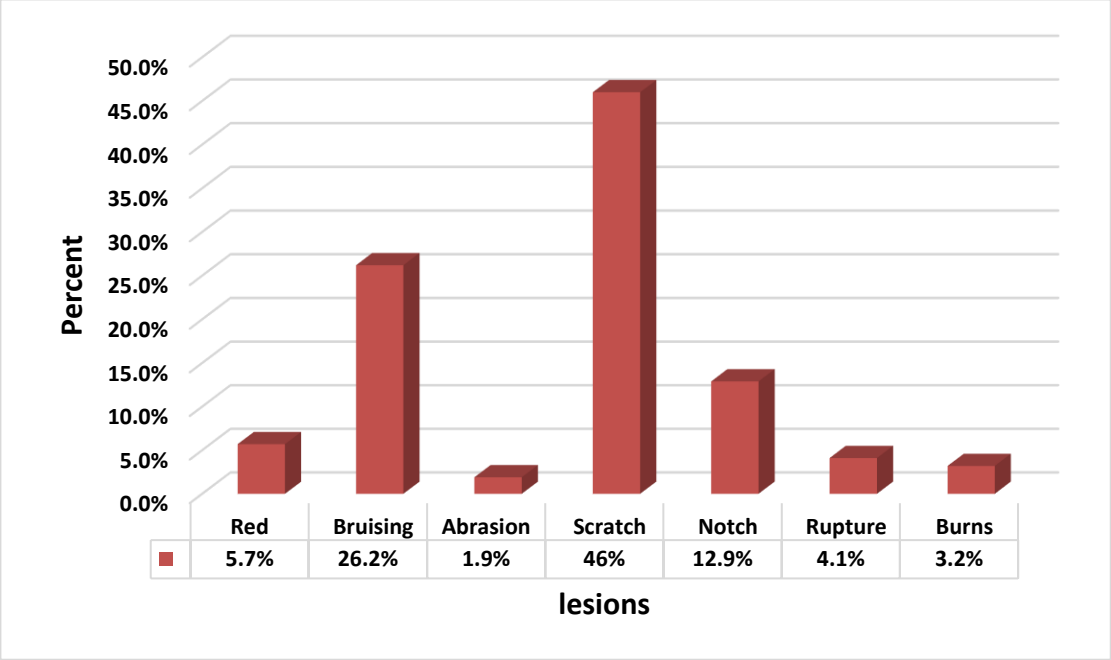

Figure 1. Frequency distribution of lesions generated by self-harmers.

The mean number of lesions was 1.52 82 0.82, while the lowest number of lesions in individuals was 1 and the highest number of lesions in subjects was 4. Also, a higher percentage of people had used hard-edged objects for self-harm (38.4%) (Table 2).

Table 2. Frequency distribution of the number of lesions created by self-harming individuals.

Number of lesions	One lesion	Two lesions	Three lesions	More than Three lesions	Total
Number	507	170	75	31	783
Percent	64.7	21.7	9.6	4	100

Discussion

In 2004, the Institute for Healthcare Improvement defined self-harm as "the expression of a personal stress that is usually in the private environment and in the form of self-harm" and divided it into three types: self-harm (including behaviors such as cutting, burning, and swallowing). Substances, hanging, jumping from heights or in front of vehicles, shooting

oneself and plunging objects into the body), poisoning (drug overdose or ingestion of toxic substances) and risky behaviors (smoking), Overeating or excessive hunger, etc.) has been divided (9).

In the present study, which examined self-harm and some related factors in patients referred to the medical organization, 783 people were diagnosed with self-harm, 59.6% of whom were men. The mean age of the subjects was 28.25 ± 8.38. Most of the subjects were in the age range of 21 to 40 years. These results were similar to the Diggins study and different from the Lee study in South Korea with a mean of 41.29 ± 17.61 (8, 10).

Most of the cases were unemployed (356 people equal to 45.5%), without higher education (57.1% less than diploma education) and married (50.6%). Most of the subjects were unemployed (356 people equal to 45.5%), without higher education (57.1% less than diploma education) and married (50.6%). In a Mars et al. Study, it was reported that the rate of self-harm was higher in people with low levels of education (11). 185 subjects (23.6%) reported a history of mental illness. In Lee's study, 10% of patients reported a history of psychiatric counseling (10). In another study, 4.7% had a previous history of self-harm. This was also reported in the study by Lee et al. With a previous history of self-harm is 85.1%. On the other hand, studies have shown that a history of self-harm is a risk factor for suicide in these people (10, 12). The majority (67.3%) of the subjects were right-handed and the frequency of self-inflicted lesions on the left side of the body (61.4%) was higher. This case is predictable due to the natural pattern in society and the dominance of the right hand as the dominant hand of individuals and on the other hand, the natural use of the dominant hand to perform various tasks.

The most common types of lesions diagnosed were scratches (46%) followed by bruises (26.2%), cuts (12.9%) and the rest. Regarding the distribution of self-inflicted lesions in the present study, the arm was in the next position with 25.7% of the most injured area and the forearm (19.9%). 64.7% had only one self-inflicted injury. The sharp and winning body (20/1) had the most wounds after the hard-edged body (38.4) and the hard body (20/4). In another related study, abrasions followed by bruising, redness, cuts, and abrasions were self-inflicted lesions, respectively, and the hard-edged body accounted for 50% of the penis used. In this case, the arm was the most injured and the forearm and neck were next.

Superficial cuts and scratches are the most common lesions of self-injury and were mostly observed in the upper extremities of the non-dominant hand, especially the forearm and wrist. In Lee's study, after intoxication and stabbing, self-mutilation followed in the third place, followed by hanging. Suicide and self-mutilation practices vary from country to country, depending on laws, culture, and economic status. In the United States, firearms are used, in rural areas of developing countries, pesticides are used, and in countries such as Korea and Japan. Medication overdoses are the most common method (10, 13).

In conclusion, in dealing with self-harm, two things must be considered: From the perspective of forensic science to differentiate the damage caused by conflict and self-harm. Therefore, a proper history and examination of how the injury occurred is very important. On the other hand, the need for psychological examinations in suspicious cases can open the door for decision-making physicians.

Author contribution

MRT and AShA wrote and completed the manuscript.

Acknowledgments

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Conflict of interest

The authors declare that they have no conflicts of interest.

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Regulating and changeable performance of *CDX2*, *CTNNBIP1*, and *FAT4* genes in colorectal cancer

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Abstract

Introduction: Colorectal cancer (CRC) is the third most frequent type of cancer in the world. In this explanation, genetic variation is associated in all cancers, particularly CRC, and modifications of numerous genes, such as *CDX2*, *CTNNBIP1*, and *FAT4*, are linked to tumorgenesis in CRC. As a result, this research was conducted in order to determine changes in the expression of these genes.

Materials and Methods: After obtaining patient consent and pathology department approval, from72 individuals with confirmation of pathology report,were provided and bought from the Bio banks. Real-time PCR was used to examine the expression of *CDX2*, *CTNNBIP1*, and *FAT4* genes in tumoral and non-tumoral tissues. These genes' histological associations with grading and staging for upregulation and downregulation were examined.

Result: *CDX2* (P = 0.01) and *CTNNBIP1* (P = 0.03) expression were highly increased, whereas *FAT4* (P= 0.05) expression was downregulated. Similarly, there was no evidence of a link between *CDX2* and *CTNNBIP1* overexpression and grade, stage, lymphnode metastasis, or distant metastasis. Furthermore, *FAT4* expression was linked to highe stage, high grade, distant metastasis and lymphnode metastasis (P 0.05).

Conclusion: *CTNNBIP1* and *CDX2* genes were upregulated in tumoral tissues, while *FAT4* genes were downregulated. Finally, changes in the expression of these genes can be used as a CRC biomarker.

Keywords: Colorectal cancer, Genes fluctuation, Regulation

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Introduction

Colorectal cancer (CRC) is one of the most commonly diagnosed cancer in adults. The third prevalent cancer in the world is CRC (1). CRC is a prevalent human cancer that requires a thorough knowledge of its molecular underpinnings. Initial therapy only cures a small percentage of people and is most effective when the disease is in its initial stages (2). CRC was among the first large epithelial malignancies in which molecular changes were observed systematically as the disease progressed. The discovery of new oncogenes and tumor suppressors would help us identify the biology of CRC and could lead to new effective treatments (3).

Since *CDX2* mutations are extremely rare events in CRCs, we hypothesized that epigenetic changes, such as promoter hypermethylation or histone deacetylation could be responsible for significant downregulation or absence of *CDX2*, particularly in the group of tumors displaying "serrated" molecular features. Human serrated adenomas with high-grade dysplasia have been shown to have significantly greater frequencies of *CDX2* hypermethylation than other polyp types (4). *CTNNBIP1* (β-catenin interacting protein 1) gene is an antagonist of Wnt signaling which binds to the β-catenin molecules. The *CTNNBIP1* function as a tumor suppressor gene or oncogene in different types of cancer is controversial. Several nuclear antagonists are known to regulate β-catenin-TCF mediated transcription. One such direct nuclear antagonist is *CTNNBIP1* (catenin, beta interacting protein 1; also known as ICAT) (5). *CTNNBIP1* binds to two different armadillo regions of β-catenin through its N-terminal and C-terminal domains leading to disruption of β-catenin-TCF interaction. The importance of *CTNNBIP1* in embryonic development and tissue differentiation process has been reported. Variable frequencies of expression of *CTNNBIP1* have been shown in metastatic and nonmetastatic human melanoma (6). The Fat gene family was originally identified in Drosophila as a member of the cadherin super-family with tumor suppressor functions. It regulates cell proliferation and planar cell polarity during Drosophila development by the Hippo signaling pathway. They encode a type 1 transmembrane protein with 34 cadherin repeats, 4 epidermal growth factor (EGF)-like repeats, a transmembrane domain and a cytoplasmic domain that is distinct from the classical cadherin proteins. In humans, four members of the Fat family have been identified, namely, FAT1, FAT2, FAT3 and *FAT4*, which are structurally similar to the Drosophila Fat protein. In mammals, *FAT4* is the true structural ortholog of the Drosophila FAT. *FAT4* functions as a tumor suppressor and previous findings have demonstrated that *FAT4* can inhibit the epithelial-to-mesenchymal transition (EMT) and the proliferation of gastric cancer cells. However, few studies have investigated the role of *FAT4* in the development of colorectal cancer (7).

Materials and Methods

Samples collection

The study sample consisted of 72 tumoral and 72 non-tumoral (margins tissues) from 53 females and 19 males were provided and bought from the Bio banks. Information on histological status is shown in Table 1. Then, all tissues were delivered to liquid nitrogen for deep freezing. Tissue samples were kept at a temperature of 80 °C for long-term conservation and investigation. Trizol (Invitrogen cat no 15596-025, USA.) was used to isolate RNA from tissues. The spectrophotometer (TC100, USA) was used for quantitative RNA analysis and electrophoresis (2% agarose gel) was used for qualitative analysis.

cDNA was prepared using the cDNA Kit (Quanti Test Reverse transcription kit, Qiagen) with around2 pg RNA per reaction. The first cDNA strand was generated utilizing a stem-loop sequence-specific primer. Table 2 lists the forward and reverse primer sequences. The real-time PCR assays were carried out on cDNA by using the SYBR Green technique in Step one equipment (Applied Biosystem, USA). A total of 1 liter of cDNA from each tissue was used for amplification. As a housekeeping gene, GAPDH (glyceraldehyde 3-phosphate dehydrogenase) was employed. Early incubation at 95 °C for 5 minutes was proceeded by 40 cycles of 95 °C for 30 s and 60 °C for 1 min in a 20 l final volume. Using the 2-ct approach, the range of up-regulation or down-regulation in each sample was extensively studied. All of the reactions were carried out in triplicate.

Table 1. Sequences of primers employed for Real-time PCR action.

Primer sequence (5′–3′)	
Forward *CDX2*	5′-TAGTTTGYGGGGYTGYTGTA-3′
Reverse *CDX2*	5′-GCCATATACRTAARCTACCTCCT-3'
Forward *CTNNBIP1*	5′-GGAAGATGGGATCAAACCTGA CAG-3′
Reverse *CTNNBIP1*	5′-TCGTATCCAGTGCTGCGACCGTAT GGATGTGTCTGCGGCGTTTTATCATG CACTGGATACGAC AAC GCCATCA CC-3′
Forward *FAT4*	5'-ACACTGTGATTGCCAGGAGAG-3'
Reverse *FAT4*	5'-GGATGTGTCTGCGGCGTTTTAT CATGCACTGGATACGACCAAGAGTC CAGTC-3'

Statistical Analyses

All the acquired data from Real-time PCR were analyzed by exercycle set. Correspondingly, the significant difference was statistically interpreted by paired Student's t-test. $P < 0.05$ was considered statistically significant. Analyses were accomplished using commercially available statistical software (SPSS Statistics software, version 25, Chicago).

Results

Gene expression evaluation in tumoral tissues

The analysis of expression levels of tumoral and corresponding non-tumoral tissues for *CDX2*, *CTNNBIP1* and *FAT4* genes indicated that the *CDX2* and *CTNNBIP1* were upregulated in tumoral tissues in comparison with their non-tumoral counterparts. On the contrary, *FAT4* expression level had decreased significantly in 50% of samples (Figure 1, 2,3).

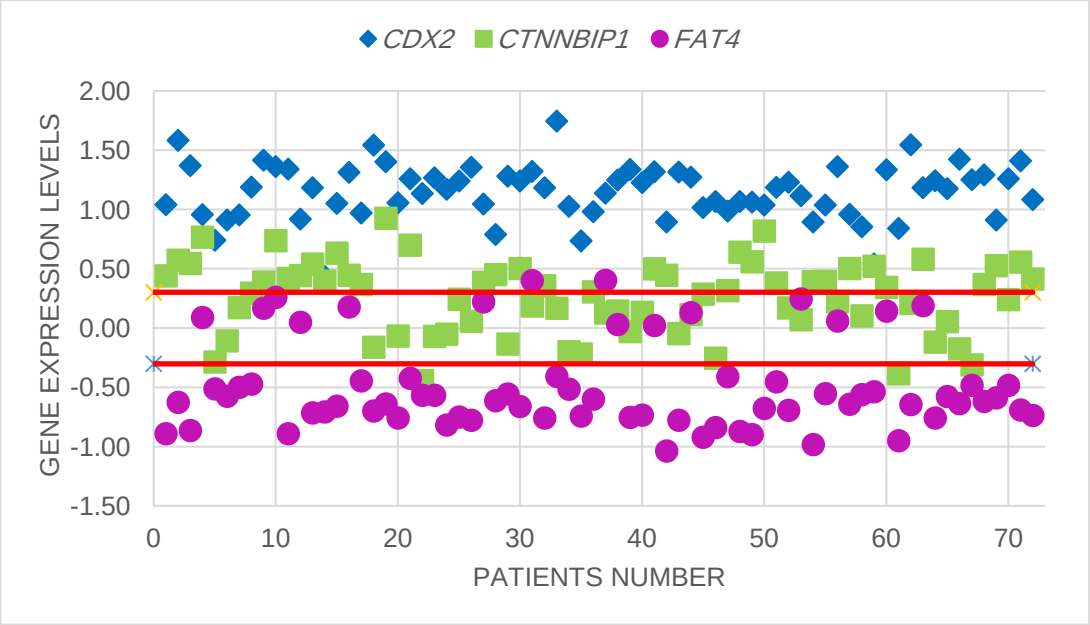


Figure1. Scatter plot analysis of relative expression of *CDX2*, *CTNNBIP1* and *FAT4* in colorectal cancer patients. The Y-axis indicates the logarithm of relative gene expression. Horizontal red lines represent cut-off values logarithms for two-fold changes in expression (FC≥2.0, p<0.05). The upper part of the graphs indicates up-regulation in the tumoral compared to the non-tumoral tissue; the lower part of the graph indicates down-regulation in the tumoral compared to the non-tumoral tissue (differences in expression ≥ 2; $P < 0.05$). The *CDX2* ($P = 0.01$) and *CTNNBIP1* ($P = 0.03$) expression level had increased and *FAT4* ($P = 0.05$) expression level had decreased significantly in tumoral compared to the non-tumoral samples.

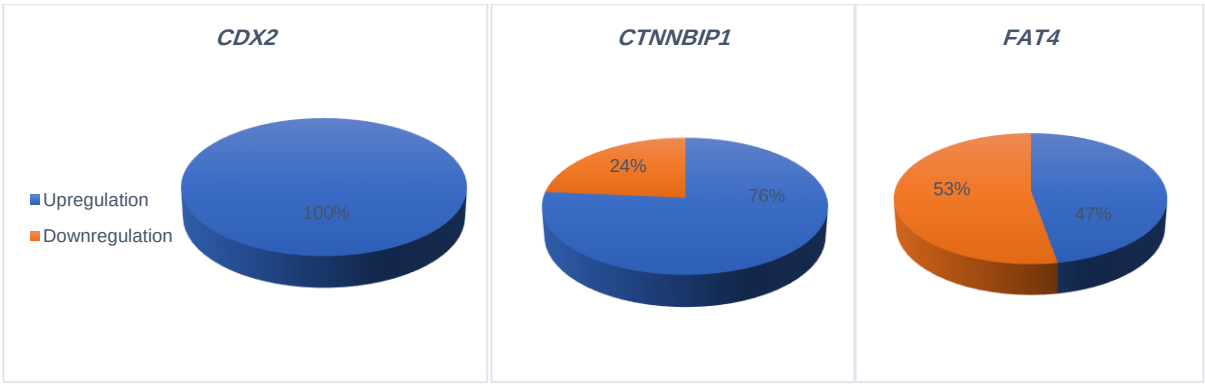


Figure 2. The data revealed a significant upregulation of *CDX2* and *CTNNBIP1* expression and downregulation of *FAT4* in colorectal cancer (P < 0.05.

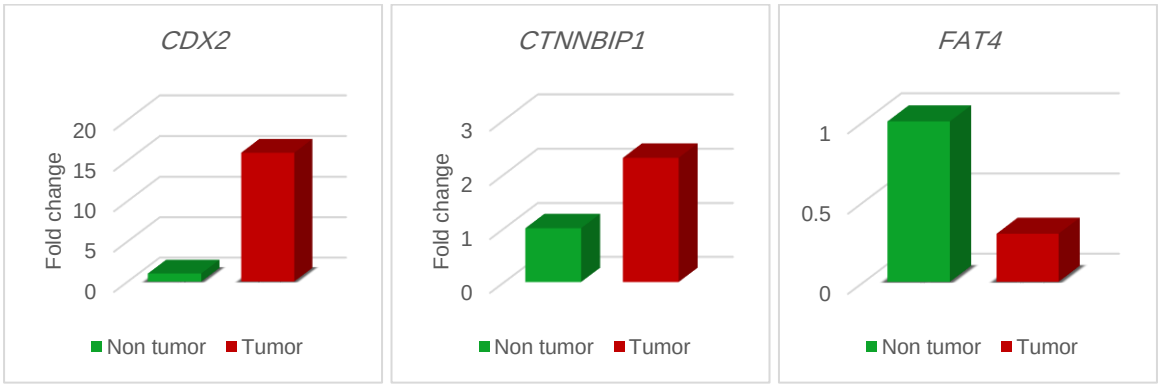


Figure 3. Fold change of **(a)** *CDX*2 (P= 0.02), **(b)** *CTNNBIP1* (P= 0.02) and **(c)** *FAT4* (P= 0.04) expression in tumoral tissues in comparison with non-tumoral (tumor margin) tissues.

Clinicopathological analysis

Clinicopathological consequences of *CDX2*, *CTNNBIP1* and *FAT4* genes expression were evaluated in 72 patients diagnosed with adenocarcinoma of the colorectal. Patients' clinicopathological characteristics are summarized in Table 2. The analysis of different clinicopathological variables and genes expression correlation is presented in Table 3 (up/down). The mean age of patients was 58.9±12.5 years at the time of diagnosis (female to male ratio, 4:1; age range, 37–88 years). In general, more than half of the patients had advanced T-stage (Stages III-IV), and high-grade histology. Lymph-node metastasis and distant metastasis were observed in more than 60% of the patients.

The number of gene expressions of all samples was compared and investigated with the stage, grade, lymph node metastasis and distance metastasis of all patients.

The analysis of different clinicopathological variables and genes expression correlation is presented in Table 3. Statistical analyzes were performed with using SPSS 25 and also Chi Square test and T test.

The expression of *CDX2*, *CTNNBIP1* and *FAT4* was matched with different clinicopathological data of the colorectal cancer patients (summarized in Table 2). There was no significant association between *CDX2* and *CTNNBIP1* expression with grade, stage, lymph-node metastasis (P= 0.02) and distant metastasis. Moreover, the *FAT4* expression was also significantly associated with high grade (P = 0.03), high stage (P = 0.03), lymph-node metastasis (P= 0.05) and distant metastasis (P= 0.05) (Figure 4, 5, 6).

Table 2. Clinicopathological characteristics of colorectal cancer cases.

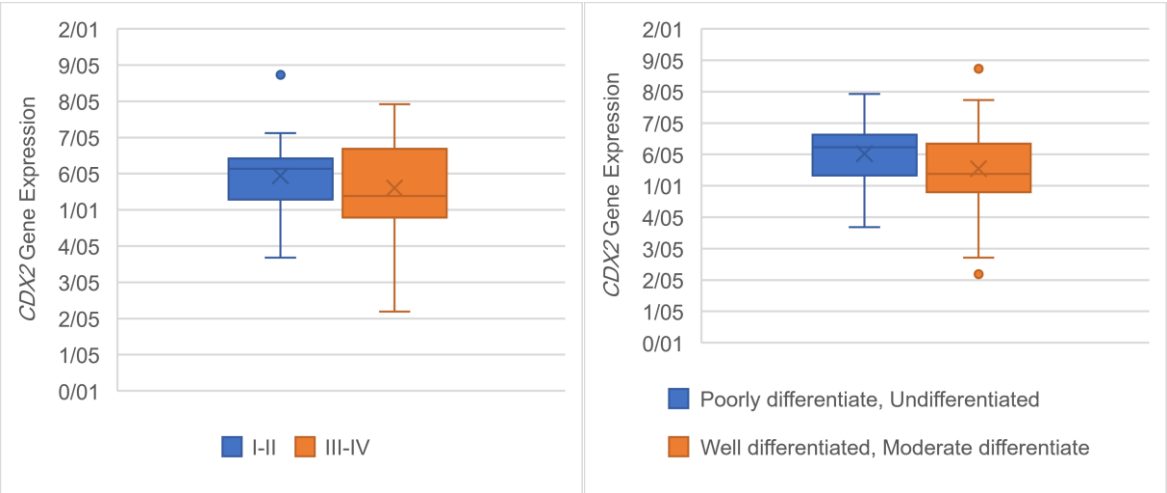
Characteristics	Total (N=72) Patients (%)
Gender	
Female	53 (73.6)
Male	19 (26.4)
Age	
< 60 years	38 (52.8)
≥ 60 years	34 (47.2)
Stage	
I	6 (8.3)
II	24 (33.3)
III	38 (52.8)
IV	4 (5.6)
Grade	
Well-differentiated	4 (5.6)
Moderate differentiate	26 (36.1)
Poorly differentiate	39 (54.1)
Undifferentiated	3 (4.2)
LM	
Yes	45 (62.5)
No	27 (37.5)
DM	
Yes	44 (61.1)
No	28 (38.9)

Table 3. The association of genes expression with clinicopathological qualification. LM: Lymph node Metastasis, DM: Distance Metastasis; ↓/−: decrease or no change of expression; ↑: increase of gene expression.

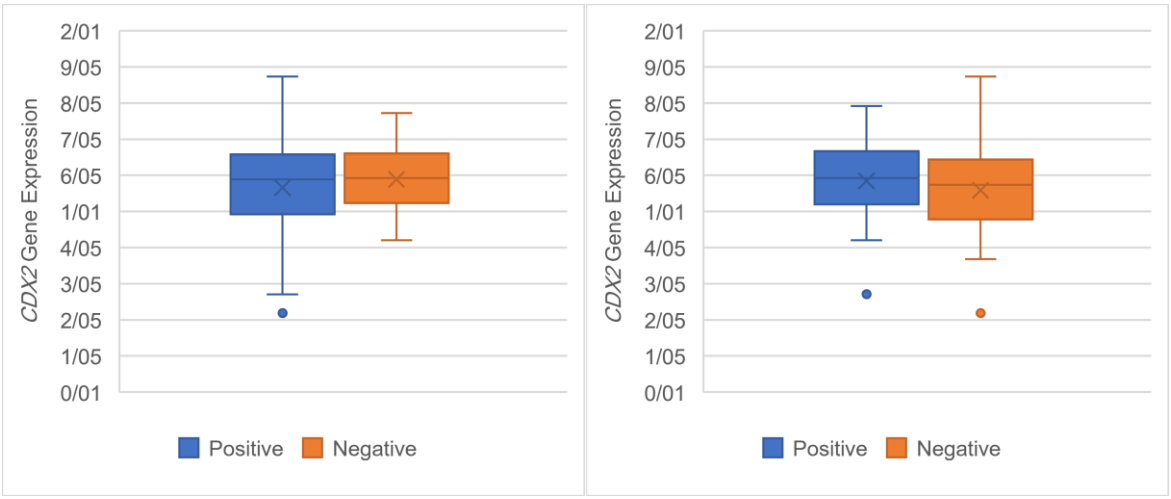
	CDX2		P-value	*CTNNBIP1*		P-value	*FAT4*		P-value
Tumor Stage	↓/−	↑		↓/−	↑		↓/−	↑	
I-II	0	30	0.5	12	18	0.7	25	5	0.03
III-IV	0	42		5	37		32	10	
Tumor Grade									
I-II	0	30	0.6	13	17	0.1	23	7	0.03
III-IV	0	42		6	36		35	7	
LM									
Yes	0	44	0.3	24	22	0.4	36	8	0.05
No	0	28		11	15		20	8	
DM									
Yes	0	44	0.2	21	23	0.5	36	8	0.05
No	0	28		15	13		21	7	

LM: Lymph node Metastasis, DM: Distance Metastasis

The Association of *CDX2*, *CTNNBIP1* and *FAT4* expression with clinicopathological qualifications

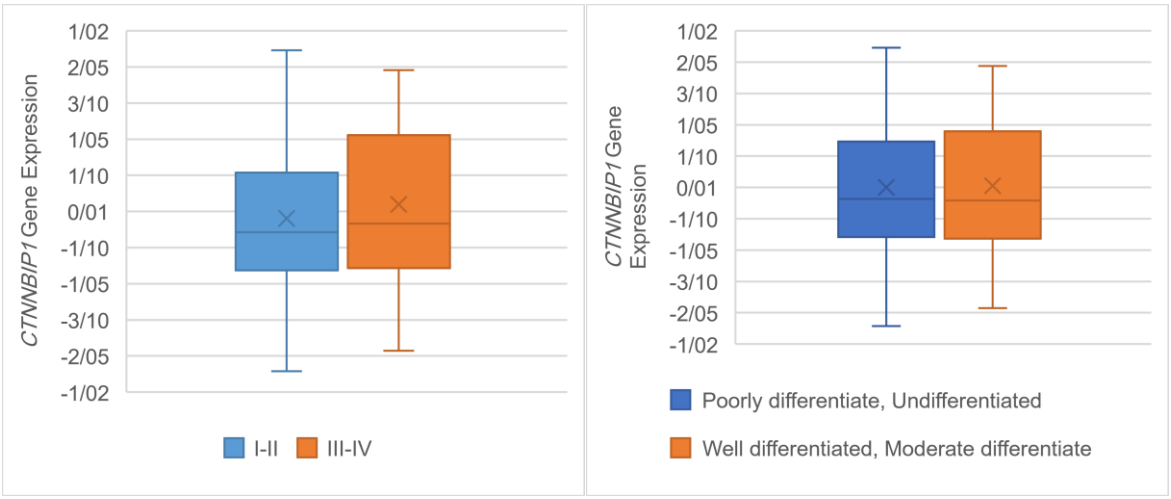


(a) **(b)**

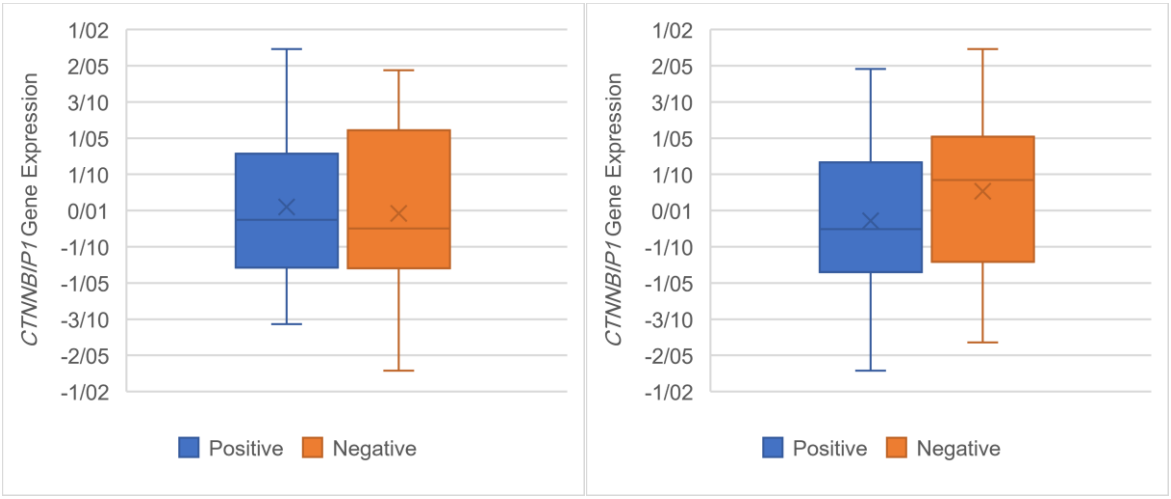


(c) **(d)**

Figure 4. The Association of *CDX2* expression with clinicopathological qualifications. There was no significant association between *CDX2* upregulation with **(a)** tumor stage (P =0.5), **(b)** tumor grade (P =0.6), **(c)** lymph-node metastasis (P= 0.3) and **(d)** distance metastasis (P= 0.2).



(a) **(b)**



(c) **(d)**

Figure 5. The Association of *CTNNBIP1* expression with clinicopathological qualifications. There was no significant association between *CTNNBIP1* downregulation with **(a)** tumor stage (P =0.7), **(b)** tumor grade (P =0.1), **(c)** lymph-node metastasis (P= 0.4) and **(d)** distance metastasis (P= 0.5).

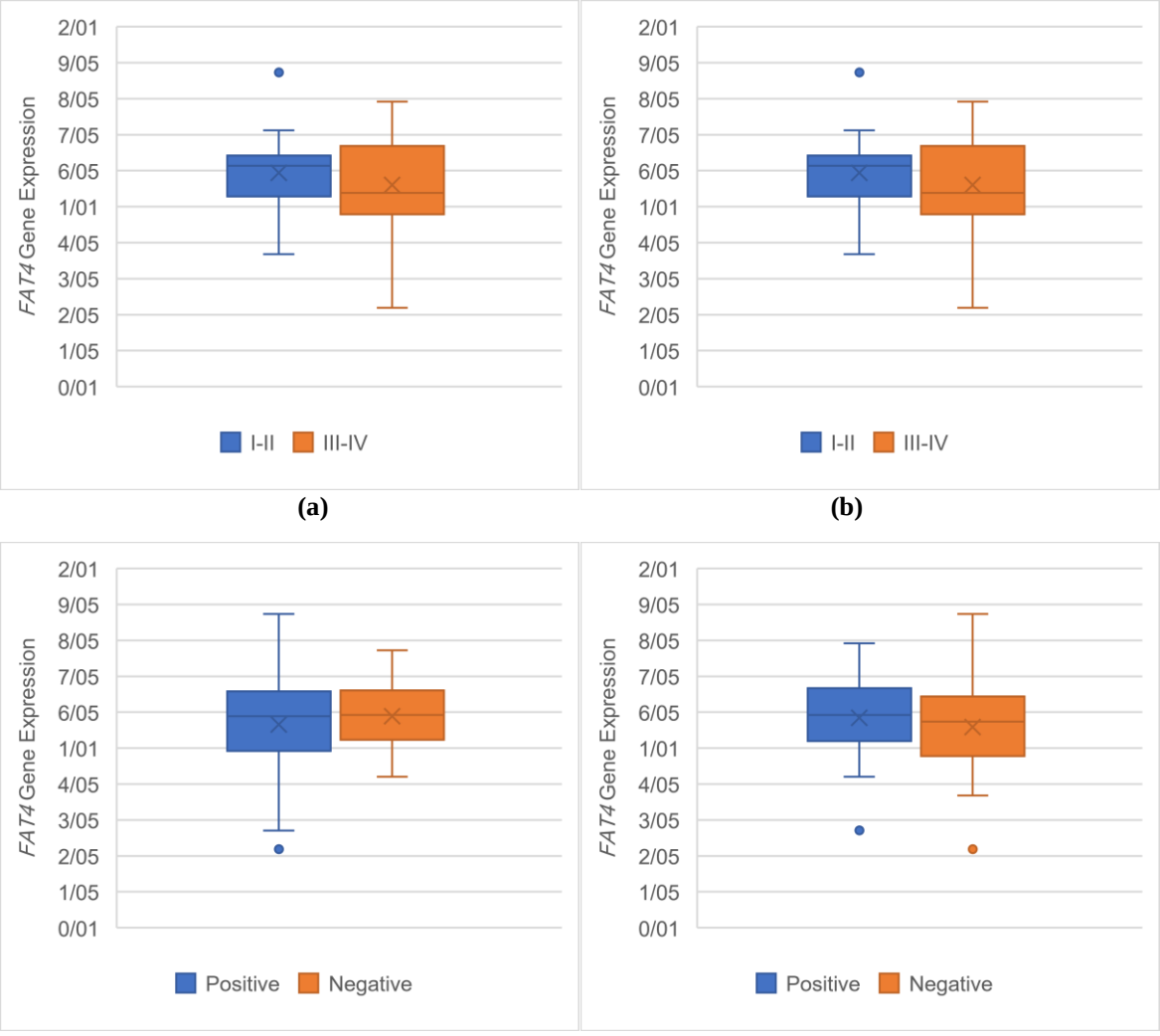


Figure 6. The Association of *FAT4* expression with clinicopathological qualifications. The *FAT4* expression was significantly associated with **(a)** tumor stage (P =0.03), **(b)** tumor grade (P =0.03), **(c)** lymph-node metastasis (P= 0.05) and **(d)** distance metastasis (P= 0.05)**.**

Discussion

Reduced *CDX2* protein expression is related to certain molecular alterations during colorectal tumorigenesis. Previous work shows that nearly all sporadic microsatellite unstable (MSI) cancers show some degree of loss of the protein in the tumor, whether in a small or substantial percentage of cells. This loss is not however limited to MSI-high cancers but is also found in microsatellite stable (MSS) tumors with BRAF mutation and high-level CpG island methylator phenotype (CIMP), in other words, in cancers deriving from the so-called serrated pathway (4). The previous research showed *CDX2* expression was increased significantly in gastric cancer. *CDX2* expression had a significant correlation with TNM stage and lymph node metastasis.

Previous findings have shown that transfection of *CDX2* cDNA, and human HT29 CRC cell line to

express *CDX2* protein, indicated the oncogenic potential of the abovementioned cells, and metastasis of related cells markedly decreased while cell sensitivity for apoptosis significantly increased. The results have shown that in comparison to the normal population, the degree of methylation of the promoter region of *CDX2* in lesion tissue of patients with CRC was higher than that of the normal population. The protein expression in the control and lesion sections of CRC patients showed that the expression level of *CDX2* in the lesion section of patients with CRC was lower. This finding suggested that there was a certain correlation between *CDX2* and CRC or the decrease in the degree of *CDX2* gene promoter methylation to a certain extent, promotes the risk of CRC (8).

Previous research indicates the downregulation of *CTNNBIP1* gene which corresponds to a tumor suppressor role for *CTNNBIP1* in GC. Also, the expression level of *CTNNBIP1* was extremely lower in female patients than males. According to our findings, the tumor-suppressing function of *CTNNBIP1* in GC is mostly associated with initiation procedures, because well-differentiated tumors showed significant downregulation of *CTNNBIP1* compared with other malignant grades. *CTNNBIP1* expression associated with EBV and CMV infections suggests that the Wnt/β-catenin dysregulation is affected by these agents in GC.

CTNNBIP1 is a suppressor of lung cancer progression. The *CTNNBIP1* protein is important, in that it can control lung cancer cell migration via the coordinated regulation of the β-catenin pathway. A low expression of *CTNNBIP1* is correlated with a high level of expression of MMP7, and there is also an upward trend in terms of the pathological stage and poorer patient survival, which suggests that *CTNNBIP1* may be able to serve as a prognostic biomarker for lung cancer (9).

FAT4 is a tumor suppressor in CRC. Moreover, *FAT4* silencing inhibits CRC cell autophagy and stimulates the invasion and migration of these cells as well as the EMT, whereas the overexpression of *FAT4* yields the opposite results and increases autophagy. Furthermore, the stimulatory effects of *FAT4* on autophagy occur through the upregulation of LC3 and the downregulation of P62 and the effects of *FAT4* on the EMT, as evidenced by the detected changes in the expression levels of E-cadherin and Twist1. Moreover, an increase in *FAT4* leads to a reduction in xenograft tumor growth in vivo, whereas the opposite outcome was obtained with *FAT4* knockdown. Therefore, we conclude that *FAT4* regulates the activity of PI3K to promote autophagy and inhibit the EMT, and these effects are partly achieved through the PI3K/AKT/mTOR and PI3K/AKT/GSK-3β signaling pathways. We anticipate that this study will provide a basis for establishing new strategic approaches for the development of effective CRC therapies (10).

Cai et al, found that *FAT4* has a tumor suppressor role mediated by the modulation of Wnt/β-catenin signaling, providing potential novel targets for the treatment of gastric cancer (11).

Conclusion

The overexpression of CDX2 and *CTNNBIP1* expression in tumoral tissues, as well as the downregulation of *FAT4*, were found to be outstanding. Interestingly, changes in the expression of these genes can be used as a primary biomarker in CRC.

Author contributions

RZ, PR, and FAS collected data and accomplished some sections of the study and manuscript, SMTH collected all the biopsies directly in Omid clinic and hospital by himself and also confirmed the clinical qualifications of all the patients as a gastroenterologist. ZKK controlled and confirmed the data quality, evaluated and optimized the informatics database, wrote the paper and edited it, some other essential functions containing study design, controlling the project and protocol development and also data analysis. All authors revised the article carefully, read and acknowledged the final version of the paper.

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Conflict of interests

Authors declare no conflict of interest.

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Evaluation of the quality of informed consent in patients referring to infertility centers of Rasht in 2019

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Abstract

Introduction: Infertility refers to couples' inability to get pregnant after at least one year of intercourse without the use of contraceptives. Getting an informed consent as one of the principles of patients' rights, is a process that she decides on therapeutic intervention after receiving adequate information from the therapist team. The lack of understanding and compliance with this process today has involved many physicians and other medical staff and hospital practitioners in the legal and regulatory authorities.

Purpose: The aim of this study was to explain the quality of the informed consent process of patients Referred to infertility treatment centers in Rasht 1398.

Materials and Methods: This study is a cross- sectional and analytical study on 172 patients referred to Alzahra infertility center in Rasht in the first half of year 1398. Patients information were collected from a questionnaire. Then data were analyzed by SPSS. Mean and standard deviation indices with 95% confidence interval were used to describe the data and Shapiro- Wilk test and Spearman's rank correlation coefficient were used for data analysis.

Result: The mean score of "providing information to the patient" with a mean of 7.23 in intermediate condition, the mean score of "understandable consent form" with 3.51 out of 4 in excellent condition and the mean score of "communicating with the physician" with an average of 9.81 were good but the "voluntary consent form" with a score of 0.6 out of 8 showed this dimension to be weak. Overall, the quality of consent of patients referred to infertility centers in Rasht with average score of 21.16 was estimated. Only weak significant relationship was found between the level of educators' satisfaction with the quality of informed consent.

Conclusion: The quality of providing information and how to get a written consent was intermediate. Therefore, it is necessary to elaborate on other available therapies and their benefits and potential complications.

Keywords: Infertility, Informed consent, Quality, Get pregnant

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Introduction

Infertility refers to a couple's inability to get pregnant after at least one year of intercourse without the use of contraceptives (1). According to the World Health Organization (WHO), 10 to 15 percent of couples in the world (more than 80 million) suffer from infertility (2) and in Iran, about a quarter of Iranian couples experience primary infertility during their life of marriage (3).

Patients, as one of the socially vulnerable groups, are supported by a set of laws aimed at observing the physical, mental, spiritual, and social needs of the patient, which are called patients' rights (4, 5), And one of the issues in the new rights of patients is the issue of informed consent in medical practices (6).

Informed consent is the process by which a patient or legal representative understands and agrees to a treatment plan (7). Self-informed consent includes the three basic components of information sharing, decision-making ability, and the ability to make free and voluntary choices (8).

Among the undeniable reasons for the need for informed consent as part of the patient's rights, there is a significant relationship between obtaining the desired informed consent (consent with sufficient awareness) and obtaining the appropriate clinical outcome including improving mental health, relieving symptoms and pain, improvement in patient's function, and physiological criteria (9). The main purpose of obtaining consent is not to reduce the physician's responsibility but to help the patient make the best decision (10).

The lack of understanding and observance of this process today has involved a large number of physicians and other medical and hospital staff and has caused a lot of material and moral damage (11, 12).

Among the studies conducted in this field are the study of Barzegar and his colleagues, which examined the level of awareness of consent obtained from patients undergoing gynecological surgery at Hazrat Zeinab Hospital in Shiraz in 2016. According to the information obtained, 43.5% of patients had an inadequate understanding of the information provided to them. The number of information patients had about their rights in the hospital and the amount of reading the consent form was in the most inappropriate situation. The findings of this study indicate the inadequate status and low awareness of consent obtained from patients undergoing gynecological surgery in Hazrat Zeinab Hospital (13).

Also, in another study, Meysami and his colleagues conducted a descriptive-analytical study on 120 people to explain the quality of the informed consent process of patients admitted to the surgical wards of a military hospital in Tehran and provide solutions to improve it in 2016. In this study, a questionnaire in the form of 19 questions was used. In evaluating the quality of informed consent of patients admitted to surgical wards, the average score of "providing information" with 18.93, "observing patients' decision-making competence" with 7.48, and "how to obtain written consent" with 5.47, is lower than the expected mean and mean scores of "Patient Perception" with 9.77, "Patient volunteering" with 8.16 and "Physician-Patient Interaction" with 16.02 were acceptable. In this study, the quality of the presenting information and the way of obtaining written consent was lower than expected and the mean score of patients' understanding, being volunteer, and interaction between physician and patient was acceptable (14).

So far, no study has focused on the quality of consent obtained from patients referred to infertility centers. This study examines the quality of informed consent of patients referred to infertility centers in Rasht.

Materials and Methods

The present study is a cross-sectional analytical study that was performed on patients referred to Al-Zahra Infertility Center in the first 6 months of 1398. The sampling method was simple random and the information of this study was collected using a questionnaire attached at the end of this thesis, as a self-report. This questionnaire has been used in Sheikh Taheri's study on hospitalization procedures under surgery (33). The validity of the questionnaire was assessed using the content application method and with the opinion of 10 professors and experts of the university, whose CVI value was 0.9 and its CVR was 0.8. The reliability of the questionnaire in the present study using the Cronbach's alpha method after completion by 35 people who refer to infertility centers in Rasht is 0.87.

This questionnaire has two parts. The first part includes demographic information of patients such as age,

waiting time for fertility, education, religion, and their number of marriages, and the second part (main) includes 22 questions in 4 areas of providing information to the patient, comprehensibility of the consent form, voluntary and doctor's interaction with the patient. The questions' scores are based on the answers yes (2 points), to some extent (1 point), and no (zero score). "No answer" or "I do not remember" answers are not rated. The total score of the questionnaire is 44-0. Thus, the score range of the questions was related to provided information (0-18), comprehensibility of the consent form (0-4), physician-patient interaction (0-14), and voluntariness (0-8). Scores below 25% are considered poor, between 50-25% are average, between 75-50% are good, and more than that are excellent. To collect data, after attending infertility treatment centers, we introduced ourselves to the recipients of infertility services and stated the purpose of this study and the method of work, and after obtaining their consent and assuring them about the preservation of the information, the questionnaires were completed by interview and we explained each question to the patient when needed.

The sample size with 95% confidence interval and the values (d = 0.15 p) and pi = 50% related to the consent form and using the following formula, 172 were estimated.

Results

In this study, 172 infertile women who were referred to the infertility center of Al-Zahra Hospital in the first 6 months of 1398 were studied. The minimum age of participants in this study was 20 years and the maximum was 46 years with a mean and standard deviation of 32 ± 5/48 years. The mean waiting time for fertility was 3/85 ± 5/6 years. Most participants (43%) had a diploma. All participants in this study (100%) were Shia. Among them, 167 people (97.1%) had their first marriage and only 5 people (2.9%) had their second marriage.

In terms of providing information to the patient, according to Table 1, most people (43.9%) stated that not enough information was available to them. Although most patients were satisfied with the information they received about the cause of infertility (61%), the method of infertility treatment (58.1%), and the cost of their treatment (68%), they mainly believed in the legal and jurisprudential aspects (68%), benefits (45.9%), side effects of treatment (50.6%), the reason for choosing treatment (43.6%) as well as other treatment options (77.3%) did not receive an explanation. In total, the average score of providing information was 18 points (3/194 ± 7/23), which showed that the status of providing information to patients was moderate.

Table 1. Consenters' opinions about providing information in the process of obtaining consent in patients referred to infertility centers in Rasht in 1398.

Questions	Yes	To some extent	No	I Don't Remember	No Answer	Total
Explain the cause of infertility	105(61)	44(25.6)	23(13.4)	-	-	172
Explain the method of infertility treatment	100(58.1)	49(28.5)	23(13.4)	-	-	172
Explain the legal aspects of treatment	2(1.2)	3(1.7)	117(68)	42(24.4)	8(4.7)	172
Explain the jurisprudential aspects of the treatment method	2(1.2)	3(1.7)	117(68)	42(24.4)	8(4.7)	172
The benefits of the treatment method	41(23.8)	50(29.1)	79(45.9)	2(1.2)	-	172
Side Effect of treatment	30(17.4)	52(30.2)	87(50.6)	3(1.7)	-	172
The reason for choosing this method of treatment	65(37.8)	31(18)	75(43.6)	1(0.6)	-	172
Explaining other treatment options	20(11.6)	18(10.5)	133(77.3)	1(0.6)	-	172
The cost of this treatment	120(69.8)	24(14)	26(15.1)	2(1.2)	-	172
Total	485(31.3)	274(17.7)	680(43.9)	93(6)	16(1)	1548

* The numbers in parentheses indicate the percentage.

According to Table 2, most of the consenters for infertility treatment (84.3%) mentioned that the consent form was understandable for them. In general, the average score in terms of comprehensibility of the consent form was (1/172 ± 3/51 out of 4 points), which indicates the excellent comprehensibility of the consent form.

Table 2. Consenters' opinions about the comprehensibility of the infertility treatment consent form in infertility treatment centers in Rasht in 1398.

Questions	Yes	To some extent	No	I Don't Remember	No Answer	Total
Adequacy of explaining the contents of the consent form	144(83.7)	12(7)	4(2.3)	9(5.2)	3(1.7)	172
Understanding the information of the consent form	146(84.9)	12(7)	2(1.2)	9(5.2)	3(1.7)	172
Total	290(84.3)	24(7)	6(1.7)	18(5.2)	6(1.7)	344

In terms of voluntary consent, only 4.2% of the participants in the study considered the choice of treatment to be voluntary. Most of the consenters stated that the benefits (89.5%) and side effects of other available treatments (87.8%) were not explained to them. In total, a score of 1/383 ± 0/6 out of 8 points, showed that the status of obtaining consent is poor in terms of volunteering (Table 3).

Table 3. consenters' opinions about the voluntary process of obtaining consent in patients referred to infertility centers in Rasht in 1398.

Questions	Yes	To some extent	No	I Don't Remember	No Answer	Total
Awareness of the possibility of withdrawal from treatment	2(1.2)	6(3.5)	142(82.6)	19(11)	3(1.7)	172
Possibility to choose other methods	16(9.3)	14(8.1)	139(80.8)	3(1.7)	-	172
Explaining the benefits of other treatments	5(2.9)	12(7)	154(89.5)	1(0.6)	-	172
Explaining the side effects of other treatments	6(3.5)	14(8.1)	151(87.8)	1(0.6)	-	172
Possibility to choose other methods	29(4.2)	46(6.7)	586(85.2)	24(3.5)	3(0.4)	688

As Table 4 above shows, the majority of consenters (56.2%) reported having a good physician relationship with them. Most patients reported trust in the physician (88.4%), comprehensibility (72.7%), and simplicity of physician explanations (73.8%), but 55.2% of people stated that they could not contact the doctor. In total, a score of 3/411 ± 9/81 out of 14 points indicates that the physician's relationship with patients was good.

The total quality score of informed consent of patients referred to infertility treatment centers is 6/457 ± 21/16 out of 44 points, which indicates its average quality.

There was no statistically significant relationship between the quality of informed consent and age and waiting time for fertility (P <0.05). However, a weak positive correlation was found between the quality of informed consent and education (P <0.05). Given that all participants in this study were Shia, no correlation can be found between the quality score of patients' informed consent to infertility treatment centers and religion. Due to the imbalance between the groups of first marriage (167 people (97.1%)) and second marriage (5 people (2.9%)), there can't be found any relationship between this variable and the quality of

informed consent of patients referred to the infertility centers.

Table 4. consenters' opinions about the relationship between physician and patient in the process of obtaining consent in patients referred to infertility centers in Rasht in 1398.

Questions	Yes	To some extent	No	I Don't Remember	No Answer	Total
Trust in the doctor	152(88.4)	19(11)	1(0.6)	-	-	172
Enough time to think and ask questions	74(43)	70(40.7)	28(16.3)	-	-	172
Possibility to contact the doctor	39(22.7)	38(22.1)	95(55.2)	-	-	172
Get complete answers to questions	81(47.1)	65(37.8)	26(15.1)	-	-	172
enough time for presenting the information	79(45.9)	63(36.6)	30(17.4)	-	-	172
Understandable physician description	125(72.7)	40(23.3)	6(3.5)	-	1(0.6)	172
Simplicity of the doctor's description	127(73.8)	39(22.7)	5(2.9)	-	1(0.6)	172
Total	677(56.2)	334(27.7)	191(15.9)	0	2(0.2)	1204

Discussion

The present study is a cross-sectional study that was performed on 172 infertile patients referred to Al-Zahra Infertility Treatment Center in the first 6 months of 1398. The samples were selected by simple random sampling and information was collected through a questionnaire through interviews. The results of the study showed that the dimension of "providing information" in the average state, the dimension of "ability to understand the consent form" in the excellent state, the dimension of "voluntary consent form" in the weak state, and the dimension of "doctor-patient relationship" in the good state. Among the 22 questions of this questionnaire, the best situation in terms of the quality of obtaining consent was related to the patient's trust and confidence in the doctor, and the lowest status was related to the patients' awareness of other treatment methods and their advantages and disadvantages.

In this study, 43.9% of people believed that the information provided was insufficient. In fact, most patients were satisfied with the explanations provided about the cause of infertility, infertility treatment method, and also the cost of their treatment, but they mentioned the lack of sufficient explanation about the jurisprudential and legal aspects, benefits and complications of treatment, the reason for choosing treatment, and they have not received other treatment options, which is consistent with the findings of Meysami (14) and Badsar (10). In contrast to the present study, Howlader's study showed that most patients are aware of the complications of surgery and even the possibility of death (15). However, in another study in Italy, 44.6% of patients had insufficient information (16).

As mentioned, one of the weakest topics studied in this study is to provide information about the advantages and disadvantages of treatment methods, which has the same result as the study of Butrle (17), Ne'matolahi (18), Ajorpaz (11), and Sheikh Taheri (19).

The present study showed that most of the consenters for receiving infertility treatment (84.3%) considered the consent form to be understandable, which is contrary to the results of Sheikh Taheri's research (19), but consistent with Joff's study in the United States that 86% of patients considered the consent form to be comprehensible (20).

Findings of this study showed that most of them do not consider their choice of treatment voluntary (85.2%). In other words, like the findings of Sheikh Taheri's study (19), and Muzur, patients do not receive sufficient information about available treatments and have no involvement in treatment decisions. However, 80% of patients are willing to participate in decision-making for their treatment (21).

The voluntary dimension in the present study was

examined to obtain information about other treatment options. Volunteering can be undermined by inadequate understanding, incompetence to make decisions or lack of sufficient and impartial information, patient respect for the physician, and pain or anxiety. Avoidance of coercion, temptation, and deception are also among the pillars of voluntariness (22-26) which have not been studied in this study. Also, this study was performed only on people who were referred to the centers due to infertility, and as a result, the negative response of patients in the voluntary section may be due to the lack of alternative treatment for them in some cases.

56.2% of people referred to infertility centers were satisfied with the doctor's relationship with them. In the study of Ajorpaz et al., 64.5% of patients were satisfied with the relationship with the physician (11), which is consistent with this study. In this study, most patients found the physician's explanations understandable and the time to present information was sufficient, which was observed in the study of Yaghmaei et al. in 67.3% of patients. (27).

Also, 43% of the people considered the time given to think and ask questions to the doctor sufficient. Mckeague's study also demonstrated the importance of making it possible for patients to ask questions (28). As mentioned earlier; Among the 22 questions of the questionnaire used in this study, the best situation in terms of the quality of obtaining consent was related to the patient's trust and confidence in the physician (88.4%), which is consistent with the study of Ne'matolahi (18). However, most of the participants in this study (55.2%) reported the impossibility of contacting a doctor, which was unlike Sheikh Taheri's study (19).

One of the limitations of this study was the lack of cooperation of private centers in accessing patients and their information. Therefore, this study was performed only on those who referred to Al-Zahra Infertility Center, while by examining the quality of the satisfaction of those who referred to a private center, it would be possible to examine the difference in the quality of satisfaction obtained in these two centers.

Another limitation of this study that makes it difficult for patients to provide information and understanding is the passage of time. the time between our contact with patients and the time of their referral to the infertility center to receive services is a few months, and this issue raises the possibility of forgetting the information given to the patient.

In this study, age, waiting time for fertility, religion, and multiple marriages were not associated with the quality of informed consent in this study, but a weak positive correlation was found between education level and the quality of informed consent, which is consistent with Minis studies (29), Faghanipour (30), Barzegar (13) and also Badsar (10).

Conclusion

In this study, the dimensions of "comprehensibility of consent form", "physician-patient relationship" and "providing information to the patient" are in the best condition, respectively, and "voluntary consent form" is the weakest dimension in the process of obtaining informed consent. So that the patient's knowledge of "benefits and side effects of other available treatments" as the biggest weakness and "patient trust in the doctor", as a strong point in the process of obtaining informed consent was obtained. Due to the significant relationship between the level of education and the quality of informed consent, it is suggested that the consent form be adjusted based on the level of literacy of patients. In this study, many people expressed the impossibility of contacting the doctor to resolve the ambiguity and ask their questions remotely (55.2%), which seems to have a significant effect on patients' trust in the doctor, so It is suggested that in future studies, the effect of patients' contact with physicians on the quality of informed consent should be investigated. In general, the quality of informed consent obtained in patients referred to infertility treatment centers in Rasht in 1398, has been reported as average.

Author contributions

SBMK, AM and ShD collected the date and wrote the paper. KD revised and finalized the article. All members of this article read the manuscript carefully and acknowledged it.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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The relationship of sexual health and marital satisfaction with spiritual health among women seeking mental health services

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Abstract

Introduction: Marital satisfaction is a multidimensional concept including various aspects. Sexual satisfaction is not only a factor affecting marital satisfaction but also a common cause of sexual dysfunction, disagreements, and communicational problems of couples. There is a relationship between these two interconnected variables and other variables, such as spirituality that is the most substantial one. This study was conducted to examine the relationship between sexual health and marital satisfaction with spiritual health.

Materials and Methods: Statistical population of this descriptive-correlational study included women who seek mental health services provided in the psychology and psychiatry clinic of the Faculty of Behavioral and Health Sciences. Of these women, 183 subjects were chosen using a convenient sampling method. The data obtained from Spiritual Health Questionnaire, Sexual Health Questionnaire, and Enrich Couple Scale (ECS) were analyzed using mean, standard deviation, and Pearson correlation coefficient through SPSS19 software.

Results: There was a positive and significant correlation between spiritual health and marital satisfaction (P-value=0.001, r=0.31) while there was not any significant association between spiritual health and sexual health.

Conclusion: Mental health issues can affect the relationships between many variables, including spiritual health, marital satisfaction, and sexual health. As an ambiguous relationship, the correlation between spiritual health and sexual health should be addressed.

Keywords: Mental Health, Sexual Health, Marital Satisfaction, Mental Health

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Introduction

Family is an integrated and efficient unit as a factor affecting the growth, behavior, and wellbeing of a person; hence, many social sciences study the significant role of the family in this case (1). The stability of family structure depends on the quality of the relationship between couples. Dysfunctional marital relationships or unsuccessful marriages not only threaten mental health but also endangers the survival of the family (2).

As a popular concept, marital satisfaction is used to evaluate the happiness and stability level of marriage. Hawkins defines marital satisfaction as feelings of happiness, satisfaction, and pleasure experienced by spouses when considering all aspects of their marriage (3). Marital satisfaction is a multidimensional concept, which consists of different aspects of marital relationships such as adaptation, happiness, integration, and commitment (2). Various factors affect marital satisfaction (3, 4). Sexual performance of couples and satisfaction with sexual relationships are factors affecting marital satisfaction (5).

According to WHO definition, sexual health is a state of mental, emotional, and social well-being, and not merely the absence of dysfunction or infirmity. Sexual health requires a positive and respectful approach to sexuality and sexual relationships, as well as the possibility of having pleasurable and safe sexual experiences, free of coercion, discrimination, and violence (6). Disagreements and communicational problems are reasons causing sexual dysfunction among couples. Sexual problems lead to other marital issues in relationships between couples. Marital satisfaction is associated with more sexual pleasure and satisfaction, which in turn leads to higher marital satisfaction (7). These two interconnected variables are influenced by many other variables, such as spirituality and spiritual health that are the most important ones (8, 9).

Spiritual health is defined as a state of being where an individual can deal with day-to-day life in a manner that leads to the realization of one's full potential, meaning and purpose of life, and excellence (10). An individual with higher spirituality has a higher quality of life and a more optimistic attitude towards life (11, 12). Religion and spirituality are overlapped. Religiosity includes behavioral manifestations of spirituality which are along with different actions associated with a specific religious group (13). Since religion is strongly linked to a wide range of relevant values and norms (14), some studied have shown that religious couples are more satisfied with their marriages compared to non-religious couples (15), while others have not experienced such relationships (16). Although many studies have proved the positive relationship between spiritual health and marital satisfaction (17, 18), many of these studies have been conducted on those who have mental health. Accordingly, mental health level affects all three variables studied in this paper (19-21). Hence, this study aimed at examining the relationship between spiritual health and marital satisfaction among applicants for mental health services.

According to Nelson (1990), if one believes in sexual desires as a symbol of relationship and intimacy then the close association between sexuality and spirituality will be revealed. The secret behind sexual desires is the human need for the spiritual and physical embrace of others (22). In this lieu, results of several studies have shown the positive relationship between religion and sexual satisfaction (9, 23, and 24). However, a relevant paper found a negative association between frequency of sexual relationship and spirituality or religiosity (25). Despite the presence of a close connection between religion and spirituality, these are two separate concepts so that that spirituality can be inside or outside of a religious framework (26). The positive relationship between religion and sexual satisfaction implies the positive association between spiritual health and sexual health, which may be stronger since spirituality is more personal, not a social concept rather than religion (26). Due to the research gap of this relationship, this study was conducted to investigate the relationship between spiritual health and sexual health.

Materials and Methods

This was a cross-sectional study with descriptive-correlational research type that was carried out after the ethics code of IR.IUMS.REC1396.30715 received from Iran University of Medical Sciences and Health Services. The statistical population comprised of all female applicants for health services who had referred to specialty and subspecialty clinics of psychology and psychiatry associated with faculty of behavioral

science and mental health, 2017. Of them, 226 subjects were chosen using the convenient sampling method of which 43 subjects were excluded regarding the exclusion criteria, including being single, having acute psychiatric symptoms such as illusion and delusion in the current situation. Finally, the information of 183 subjects was analyzed. After the selected subjects announced their informed consent, therapists asked them to fill out the following questionnaires:

Spiritual Health Questionnaire: this 48-item questionnaire was designed by Amiri et al. (2014) to assess spiritual health within three conceptual structures of insight, tendency, and behavior. These structures measure three sub-scales of relationship with self, god, and surroundings. Each item is scored on the Likert Scale from 1 (strongly agree) to 5 (strongly disagree). In the next step, scores are converted to 0-100 format (1-100, 2-75, 3-50, 4-25, 5-0) that the higher scores indicate a higher level of spiritual health. Internal consistency (Cronbach's alpha= 0.98) and retest reliability of this questionnaire obtained at an optimal level. Moreover, the content validity of this questionnaire was confirmed by experts (27).

Sexual Health Questionnaire: this questionnaire was developed by Rouhani et al. (28) to examine sexual health. This tool includes 33 items scored on a 3-point Likert scale, including I agree, I do not know, I disagree. This questionnaire explains more than 92% of sexual health variance with a reliability coefficient of 0.82. Exploratory and Confirmatory Factor Analyses indicated suitable and adequate validity and reliability indicators to measure sexual health.

ENRICH Couple Scales (ECS): this scale was designed by Olson in 1985 and was updated in 2010 (29). This questionnaire includes 35 items and four subscales. Alpha coefficients of marital satisfaction, communication, conflict resolution, and ideal distortion subscales equaled 0.86, 0.80, 0.84, and 0.83, respectively; retest reliability of these subscales equaled 0.86, 0.81, 0.90, and 0.92, respectively. Daneshpour et al. (2011) translated this scale into Persian and measured alpha coefficients of marital satisfaction (0.78), communication (0.78), conflict resolution (0.62), and ideal distortion (0.78) (30).

The collected data were analyzed through descriptive analysis of data, including mean and standard deviation, as well as inferential analysis including Pearson correlation coefficient through SPSS19 software. After the normality of data was examined, the Kolmogorov-Smirnov test was used for inferential analysis.

Results

Table 1 reports descriptive indicators of variables. According to Table 1, subjects were in the age range of 23-69 with an age average of 38.8. Mean (SD) of marital satisfaction, mental health, and sexual health equaled 94.03(12.84), 81.83(21.36), and 53.54 (7.90), respectively.

Table 2 reports correlation coefficients between spiritual health, marital satisfaction, and sexual health.

Table 1. Descriptive indicators of variables**.**

	Age	Marital satisfaction	Spiritual health	Sexual health
Mean	38.88	94.03	81.83	53.54
SD	8.56	12.84	21.36	7.9
Min	23	53	48	33
Max	69	149	141	70

Table 2. Correlation between variables.

		R	P-values
Spiritual health	Marital satisfaction	0.31*	0.001
	Sexual health	0.11	0.22

* Significant correlation at level of 0.01

According to Table 2, there is a positive and significant correlation between spiritual health and marital satisfaction (P-value=0.001, r=0.31) while there was not any significant correlation between spiritual health and sexual health.

Discussion

The positive side of this study is the investigation of spiritual wellness and sexual health by using a native questionnaire although there was not any significant relationship between these two variables in this paper. This finding has been discussed herein.

According to the results of the extant study, there was a positive relationship between spiritual health and marital satisfaction, which means that an increase in spiritual health leads to enhancement of marital satisfaction and vice versa. This finding was consistent with results obtained from studies conducted by Salehi et al. (2017), Tajvidi et al. (2017), and Mousavi et al. (2015) who found a positive significant relationship between spiritual health and marital satisfaction among students, married women who had referred to health centers, and nurses (8, 17, 31).

Zarei and Ahmadi (2016) found a positive and significant relationship between spiritual intelligence, which can be a subset of spiritual health, and marital satisfaction (32). Zaheri et al. (2016) conducted a systematic study and found positive effect of religious, spiritual, sexual, interpersonal, and mental health factors on marital satisfaction (33); such relationship not only seen in Iranian culture that is affected by the religious viewpoint but also can be observed in other communities (34). Farshadnia et al. concluded that married women and men have higher spiritual health (35), which provides a consistent association between internal forces. Spiritual health is associated with some characteristics, including life stability, calmness, and a sense of close relationship with God and the environment. In general, it can be stated that mental health affects the health and stability in life, in particular marital life (17). Mental health affects marital satisfaction through various mechanisms. For example, Hosseini and colleagues (2019) indicated that spirituality could strengthen the morale of social acceptance and cohesion by increasing social participation and prosperity. Moreover, spirituality provided individuals with intra-group social supports and interactions by making an empathic attitude. Accordingly, spirituality leads to higher social wellbeing with a positive and direct effect on marital intimacy (36). Another study indicated that religious attitude could predict marital satisfaction through resilience (37).

In the abovementioned studies (8, 17, 31), the correlation between spiritual health and marital satisfaction was higher than 0.60, which was greater than the equivalent correlation obtained in the present study (r=0.31). As the surveyed sample of this study comprised female applicants for mental health services and there was a significant relationship between spiritual and mental health (19), as well as between mental health and marital satisfaction (21), the relationship between spiritual health and marital satisfaction is affected by mental health. Spiritual health influences the behavior of individuals when dealing with life-related issues, and can contribute to

the improvement and promotion of psychological wellbeing (38).

According to the results of the present study, there was not any significant relationship between spiritual wellness and sexual health. Several studies have found the positive impact of religion on satisfaction with sexual life (9, 24), while some other studies have found a negative relationship between religion and sexual health (39). Such opposite results may stem from the different relationship between religion and sexual satisfaction in various groups; on the other hand, they might be associated with the use of different tools for evaluation of the multidimensional concept of religion (9). It worth noting that assessment of spiritual health was done based on the questionnaire designed by Amiri et al. who assume spirituality and religion as interconnected concepts regarding religious beliefs in Iranian society. Different religions from a specific attitude toward a sexual relationship in minds of individuals (40). Although religion and spirituality are overlapped, they are distinct concepts, which may leave different effects. Since spiritual and religious beliefs are crucial issues in the life of Iranian people, it is possible to expand the positive attitude of people towards life through spirituality (41). Rahmati and Mohebbi (2018) studied the relationship between spiritual intelligence and sexual satisfaction and found a significant association between them. They defined spiritual intelligence as a facilitator to solve daily problems and to achieve goals (42). Accordingly, problem-solving is a substantial factor associating with mental health (43). The extant paper studied the relationship between the spiritual and sexual health of applicants for mental health services. Mental health plays a mediating role in the relationship between these two variables and justifies the lack of correlation between them.

Conclusion

Sexual health and marital satisfaction contribute to the stability of the family and the health of the community. To this end, variables affecting sexual health and marital satisfaction should be addressed and required measures should be taken to improve positive effective variables. On the other hand, it is difficult to achieve this goal since it is related to mental health issues, which influence the relationship between other psychological factors. As mentioned in this study, spiritual health affected the marital satisfaction of applicants for mental health services, while spiritual health could not explain the sexual health of the studied group. Due to the research gap in the relationship between spirituality and sexual health, it is recommended to examine this relationship by consideration of other effective factors, such as mental health. The most important constraint of this study was the limited number of the studied population and the convenient sampling method that selected only married women referring to specialized and subspecialized psychology and psychiatry clinic of faculty of behavioral sciences and mental health. Hence, caution should be taken when generalizing the results of this study.

Author contribution

ZKh, NM, EF and ShGh wrote and compiled this article. MB wrote and edited the manuscript comprehensively. All authors confirmed the final version of the paper.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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The prevalence of sleep disturbances among patients with COVID-19

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Abstract

Introduction: Due to the importance of sleep disorders and the unknown effects that they may have on the course of the disease in COVID-19 patients, in this study, we aimed to investigate the factors affecting sleep disorders in these patients.

Materials and Methods: The present research was a cross-sectional analytical study conducted in Razi Hospital in Rasht. The study population included COVID-19 patients referred to the corona clinic of Razi Hospital for follow-up. Information about age, gender, body mass index (BMI), underlying disease, drugs used, a history of using cigarette and opioids, duration of hospitalization, and type of hospitalization (normal ward or the intensive care unit (ICU) ward) was extracted from patients' files and recorded in the data collection form. The Petersburg Sleep Quality Index (PSQI) was used to assess sleep disorders. The collected data were entered into SPSS software version 24. The significance level of the tests was considered $P < 0.05$.

Results: The mean age of the subjects in this study was 43.79 years. According to the results, 52 people were male (52.5%) and the rest were female. Based on the results, it was found that age ($p = 0.540$), gender ($p = 0.141$), BMI ($p = 0.464$), cigarette use ($p = 0.675$), opium use ($p = 0.757$), underlying disease ($p = 0.430$), drug use ($p = 0.327$), and duration of hospitalization ($p = 0.203$) were not significantly associated with sleep disorders.

Conclusion: According to the findings of this study, sleep in patients with COVID-19 is not associated with age, gender, cigarette use, opium use, underlying disease, duration of hospitalization, and a history of drug use.

Keywords: Sleep disorder, COVID-19, Petersburg Sleep Quality Index, Cigarette use, Opium use

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Introduction

Since early December 2019, numerous cases of pneumonia due to an unknown etiology were reported in Wuhan, Hubei Province, China (1–3). Most patients worked in the seafood wholesale market (1–4). The cause of this disease was a new and genetically modified virus from the family of coronaviruses called SARS-CoV-2, which was named COVID-19. Unfortunately, due to its high transmissibility, the virus spread rapidly throughout the world and infected almost all countries of the world in a short time (less than 14 months) (5,6) . According to official reports, more than 7 million people in the world have been infected with the virus as of June 25, 2020, and the number of deaths due to this virus has been reported to more than 400,000 (7). Coronaviruses are a group of very diverse, single-stranded RNA viruses. They cause many diseases related to the respiratory, intestinal, hepatic, and nervous systems with varying severity in humans and animals (8,9). COVID-19 is the seventh recorded coronavirus that infects humans and has 75%-80% genomic resemblance to the severe acute respiratory syndrome coronavirus (SARS-COV), 50% to the Middle East respiratory syndrome coronavirus (MERS-COV), and 96% to bat coronavirus (3). Therefore, current evidence strongly supports that the new coronavirus (COVID-19) has been derived from bats, although its intermediate hosts are not yet known (10). Studies have shown that, like the SARS coronavirus, the COVID-19 virus uses the receptor, the angiotensin II converting enzyme (ACE2), to enter the cell (3,11). The most important clinical symptoms upon arrival of COVID-19 patients at the hospital include fever, cough, sputum, headache, vomiting, diarrhea, fatigue, rhinorrhea, and chest pain. Almost 80% of patients with the new coronavirus show mild symptoms and recover at home. In 14% of cases, the sufferer shows severe symptoms, including pneumonia and shortness of breath. In 5% of cases, the patient's condition worsens, associated with respiratory failure, infectious shock, and failure in other organs of the body (11). Given the pathogenicity of the virus, its high rate of spread, and its resulting mortality rate, this disease may affect the mental health status in different strata of society as well as the patients with this disease. Sleep disorder in COVID-19 patients is one of the problems related to individuals' mental health and well-being. Patients with this disease should be isolated which the patient's isolation during the course of the disease may exacerbate anxiety and sleep disorders (12,13).On the other hand, sleep disorders can aggravate psychological stress and increase stress and defective cycle, followed by various changes in the body's immune and hormonal systems, which in turn intensify the destructive course of the disease. For example, with increasing levels of cortisol in the body, the mortality rate of patients with COVID-19 increases, showing the importance of the above-mentioned cases and one of the ways through which sleep disorders affect the disease (14). During sleep disorders, patients may also have to take soporific drugs, which these drugs through sedative mechanisms and muscle relaxation, may affect respiratory fatigue or other factors leading to exacerbating disease course and mortality. Because of the importance of sleep disorders and the unknown effects that they can have on the course of COVID-19, in this study, we aimed to investigate the frequency of sleep disorders in COVID-19 patients.

Materials and Methods

Study Population

The study population in this research included COVID-19 patients referred to the corona clinic of Razi Hospital for follow-up. Inclusion criteria were all patients with COVID-19 whose disease had been confirmed by computed tomography (CT) scan or polymerase chain reaction (PCR), and after treatment and discharge from the hospital of origin, they had referred to the corona clinic of Razi Hospital. Exclusion criteria were patients with incomplete files or those who were not willing to participate in this study.

Data Collection

The present research was conducted as a cross-sectional analytical study. Information about age, gender, body mass index (BMI), underlying disease, drugs used, a history of using cigarette and opioids, duration of hospitalization, and type of hospitalization (normal ward or the intensive care unit (ICU) ward) were extracted from patients' files and recorded in the data collection form. The Petersburg Sleep Quality Index (PSQI) was used to assess sleep disorders. This questionnaire is one of the best tools designed and developed to measure sleep quality which was

developed in 1989 by Dr. Boyce et al. at Pittsburgh Psychiatric Institute (15). To exclude patients who had insomnia due to depression or anxiety (exclusion criteria), the Hospital Anxiety and Depression Scale (HADS) was used. This scale was designed by Zigmond et al. in 1983 to measure patients' risk of depression and anxiety (16).

Statistical Analysis

In this research, descriptive statistical methods such as frequency, percentage, mean, and standard deviation were used to describe the obtained data. The Independent Samples Test was used to examine the relations of age and BMI to sleep disorders; the Pearson Chi-square Test was used to examine the relations of gender, cigarette use, underlying disease, and drug use to sleep disorders; the Fisher's Exact Test was used to examine the relationship between opium use and sleep disorders, and the Mann-Whitney U Test was used to investigate the relationship between duration of hospitalization and sleep disorders. The data analysis was performed using SPSS software version 24, and the significance level of all tests was considered 0.05.

Results

The mean age of the subjects in this study was 12.54 ± 43.79 years, among which 52 people were male (52.5%), and the rest were female. The mean BMI of the subjects was 3.22 ± 26.83. Table 1 shows the frequency of the type of underlying disease in the subjects.

According to the results, 35 people (35.4%) had a history of drug use, 35 (35.4%) had a history of cigarette use, and 15 (15.2%) had a history of opium use. The mean duration of hospitalization of the study subjects was 2.84 ± 4.81 days.

Based on the results, 71 people (71.7%) had mild sleep disorders. In addition, the analysis of the data of this study showed that age, gender, BMI, cigarette use, opium use, underlying disease, drug use, and duration of hospitalization were not significantly associated with sleep disorders.

Table 1. Frequency of subjects based on the underlying disease.

Disease	Number	Percent
High blood cholesterol	4	12.12
High blood pressure	8	24.24
Diabetes	4	12.12
Chronic heart failure	1	3.03
Chronic kidney failure	1	3.03
Hypothyroidism	1	3.03
Osteoarthritis	1	3.03
Fatty Liver	1	3.03
High blood pressure + high blood cholesterol	4	12.12
High blood pressure + chronic kidney failure	1	3.03
High blood pressure + prostate hypertrophy	2	6.06
Diabetes + hypothyroidism	1	3.03
High blood pressure + high blood cholesterol + diabetes	2	6.06
High blood pressure + diabetes	1	3.03
High blood pressure + chronic kidney failure + prostate hypertrophy	1	3.03
Total	33	100

Discussion

The COVID-19 disease is unique because it has infected all countries of the world due to its high transmissibility in less than a few months and creating a pandemic situation (12,13). Currently, the number of patients infected with this disease as well as the resulting mortality, is increasing rapidly worldwide (5,6). Accordingly, and considering the current emergency situation of this disease, it is predictable

that some of the symptoms of psychological disorders will appear in COVID-19 patients. In this regard, the results of numerous studies on COVID-19 patients in China during the disease spread, some of these psychological disorders, including anxiety, fear, depression, emotional changes, insomnia, and post-traumatic stress disorder (PTSD) with a high prevalence have been reported in these patients (17,18). According to previous studies, COVID-19 patients have low psychological tolerance, and due to the current state of the disease in the world, these individuals are highly exposed to psychological disorders such as anxiety, fear, depression, as well as negative thoughts (19). Also, according to the results of a study in China, the prevalence of low-quality sleep is 38.3%, the prevalence of problems related to sleep onset is 29.8%, and the prevalence of insomnia is 29.1% in patients with COVID-19 (20). In this study, the frequency of sleep disorders in COVID-19 patients was investigated. Based on the results, there was no significant relationship between age and sleep disorders. The results of a study in Bangladesh in 2020 showed that the prevalence of sleep disorders in COVID-19 patients in the age range of 31 to 40 years was much higher than in other age groups (21). The results of a study on COVID-19 patients in China in 2020 showed that sleep disorders were higher in patients older than 35 years (22). With increasing age, some changes occur in the quantity and quality of sleep, leading to sleep disorders and frequent complaints related to these disorders. On the other hand, the incidence of physical diseases exacerbates the incidence of sleep disorders in this age group. Elderly patients also experience issues such as retirement or the death of a loved one (for example, a spouse), leading to emotional stress. For this reason, they often have delays in falling asleep as well as frequent awakenings during sleep. In addition, the elderly often take medications to control and treat chronic diseases, which these medications can affect the quantity and quality of sleep. The elderly are more likely to develop primary sleep disorders such as obstructive sleep apnea, which may predispose them to chronic sleep disorders (23). However, based on the results, sleep disorders are not significantly associated with taking medication. The results of a study showed that the use of muscle relaxants can be effective in improving sleep quality and reducing anxiety in patients with COVID-19 (24).

In the following, according to the results, it was found that sleep disorders were not significantly related to gender. In line with the results of the present study, a study on COVID-19 patients in China in 2020 showed that the rate of sleep disorders was not related to patients' gender (22). Contrary to the results of the present study, a study in Bangladesh in 2020 showed that the prevalence of sleep disorders in COVID-19 patients was higher in women than in men (21). The results of another study in Bangladesh showed that the rate of generalized anxiety and subsequent sleep disorders was higher in women with COVID-19 than in men (25). Differences in the results of different studies may be due to differences in the demographic characteristics of the subjects. However, in previous studies, sleep disorders were generally associated with gender. For example, Kijna et al. have mentioned gender as one of the important effective factors in sleep disorders (26). and the study of Trible has also shown that being a woman is a factor in sleep disorders (27). This finding may be due to the home duties of women and their preoccupations at the time of hospitalization because of not doing housework and the affairs related to their children, which cause sleep disorders in them. Another reason which can be raised is the issue of women's menopause, which causes them to have trouble sleeping compared to their youth because, during menopause, sleep onset is delayed with frequent awakenings, and sleep time is shortened. Also, based on the results, there was no significant relationship between BMI and sleep disorders. Given the relationship between the negative effects of obesity and inactivity on sleep (28). it was expected that patients with higher BMI would have higher rates of sleep disorders, which was not the case. In line with the results of the present study, a study on COVID-19 patients in China in 2020 showed that the rate of sleep disorders was not related to patients' BMI (22). Based on the results, sleep disorders were not significantly associated with cigarette use. Consistent with the results of the present study, a study on COVID-19 patients in China in 2020 showed that the rate of sleep disorders was not related to cigarette use (22). It seems that the main factors in sleep disorders in COVID-19 patients are different from the common causes of sleep disorders in other individuals in the community. For example, it has been suggested that sleep disorders in COVID-19 patients are more psychological and stem

from anxiety. The results showed that sleep disorders were not significantly associated with opium use. Given the high prevalence of depression in patients who use opium and the role of depression in the development of sleep disorders (29). it was expected that the rate of sleep disorders in patients who use opium was higher, which was not the case. Also, based on the results, sleep disorders were not significantly related to the underlying diseases. According to a study by Basedosky et al., sleep disorders can lead to more activation of inflammatory processes and changes in the cytokine system in individuals with underlying diseases, and subsequently, these changes can exacerbate sleep disorders. The studies by Hung et al. have also shown that the mortality rate is higher in septic patients with sleep disorders. From the above articles, we can understand the possible prevalence of insomnia in COVID-19 patients and the role it can play in the prognosis of these patients (30–32). Therefore, it was expected that in patients with underlying diseases, due to higher levels of systemic inflammation, the rate of sleep disorders would be higher, which was not the case. In the following, based on the results, there was no significant relationship between the duration of hospitalization and sleep disorders. According to the results of a study by Guo et al. in May 2020, the rate of sleep disorders is higher in coronary patients due to isolation, hospitalization, and distance from family (33). According to a study by Zhang et al. in June 2020, it was found that many patients with insomnia were admitted to the ICU, but after investigating patients without sleep problems, it was found that none of them required ICU admission (34). Therefore, it was expected that the rate of sleep disorders would increase following increasing patients' duration of hospitalization, which was not the case.

Conclusion

The results of the present study showed that sleep disorders in COVID-19 patients were not associated with age, gender, cigarette use, opium use, underlying disease, duration of hospitalization, and a history of drug use.

Author contribution

MRT, RS, MA wrote the manuscript ,revised and conducted this study. PT, ShMO, SM, SAAM, FK and ShSMSh contributed in some sections of the article. All authors read the final edited version of the manuscript.

Ethical Considerations

Compliance with ethical guidelines: IR.GUMS.REC.1399.273.

Conflict of interest

The authors declare no potential conflicts of interest.

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Importance of QF-PCR method in aborted embryos in comparison with other common relative determination aneuploidies methods

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Abstract

One of the most important method in cytogenetic in order to diagnose the chromosomal abnormalities, is QF-PCR (Quantitative fluorescent polymerase chain reaction).In this way, QF-PCR can be employed in diagnosing the chromosome duplication number by amplification of repeat sequences at polymorphic loci. These repeat sequences are amplified by PCR, and the labelled yields are classified by gel electrophoresis method. Importantly, QF-PCR reaction has been in diagnostic application in many countries and has confirmed to be a robust, cost-effective, and precise rapid prenatal test for many types of common aneuploidies. Special benefits comprise detection of mosaicism, triploidy and maternal cell contamination. So, we try to declare the importance of this technique in comparison with other ones in this review article.

Keywords: QF-PCR, Aneuploidies, Abortion, Chromosomal abnormalities

Introduction

Abortion is the involuntary termination of pregnancy before the twentieth week. Although abortion is a common experience, it is not because it is easy, and it is possible to take steps to treat and prevent it by examining its causes.

Meanwhile, women who experience more than three consecutive miscarriages have recurrent miscarriages. Recurrent miscarriage as a multifactorial disease involves several issues, including immune, anatomical, hormonal, and genetic disorders. In more than 50% of cases, no cause for recurrent miscarriage is identified.

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Disruption of aneuploid chromosomes can be one of the causes of recurrent miscarriage. High-risk pregnancies for chromosomal defects have been studied for two decades by conventional cytogenetic methods. The routine cytogenetic method is very accurate in examining chromosomal defects, but the most important problem of this method is its long duration. Recently, rapid chromosomal screening methods have been developed that detect major abnormalities of certain chromosomes in just one or a few days. These methods include FISH, MLPA, and QF-PCR. Quantitative fluorescence polymerase chain reaction (QF-PCR) is a cheap, fast and reliable method for prenatal diagnosis of aneuploidy on chromosomes 13, 18, 21, X and Y (Table 1). In recent years, quantitative fluorescent technique, PCR or QF-PCR, has been used to rapidly detect chromosomal abnormalities before birth. In this method, short repeats (STRs) or markers on DNA are amplified and marked with fluorescent markers and their value is measured by electrophoresis.

Table 1. QF-PCR efficiency in cytogenetics.

Number of cases detected / number of actual nonmosaic autosomal trisomies and triploidies	Number of cases detected / number of actual non-mosaic sex chromosomal aneuploidies	Sample failure/ not tested or UI, %	Number and type of specimens	False positives for sex chromosomal aneuploidy	False positives for trisomy or triploidy
14/15 1 case of T18 was UI	5/5*	0 / 0	662 AF	0	0
89/89	16/20	0.1 / 2	5097 AF	0	0
437/437	N/A	0.09 / 2.1	7720 (6147AF, 1552 CVS 21 FBS)	N/A	0
16/19 3 cases were UI	0/0	0 / 1.2	1020 AF	0	0
14/14	1/3 Only 2 markers on X chr	0 / 2.5	687 AF	0	0
429/429	NA	2.9	10253AF	NA	0
71/73, 2 cases missed at the beginning when only two markers per chr used.	2/8	0.06 / 0.15	4692 AF	0	0
202/202	14/14	None reported	3854 AF	0	0
110/110	20 / 20	0 / 0.26 for AF	2906 (142 CVS, 2764 AF)	0	0
15/15	4/4	0 / 2.9	576 AF	0	0
1287/1290† 3 cases were UI	265/267†‡ 1 abnormality missed; 1 UI MCC	0.05 / 0.82	37544 AF 4687 CVS	0	0

AF: amniotic fluid; CVS: chorionic villus sampling; chr: chromosome; MCC: maternal cell contamination; FBS: fetal blood sample; UI: uninformative markers.

1-1 Scientific definition of pregnancy

Pregnancy or pregnancy is a condition in which a woman has an embryo or fetus in her womb. Pregnancy is also called the "pregnancy period", which ends with the birth of the baby (delivery). It is noteworthy that the word embryo is assigned during the first 9 weeks after fertilization and the word embryo is assigned from the tenth week to the end of pregnancy. In humans, a normal pregnancy lasts about 38 weeks from fertilization. If the length of this period is calculated from the last menstrual period of a pregnant woman,

the normal amount will be approximately 40 weeks. The developing human sperm in the first weeks of pregnancy is called an embryo and after this period, until the end of pregnancy, it is called an embryo. Humans usually have only one fetus in the womb at a time of pregnancy, although multiple births are not uncommon. The World Health Organization sets a normal pregnancy time of 37 to 42 weeks. In scientific terms, the state of pregnancy is referred to as gravida and to the pregnant female is gravida. A woman who has never been pregnant is called a neoliberal and a woman who becomes pregnant for the first time is called a premiere, and in subsequent pregnancies it is called a multivariate. A woman who has never been pregnant or has not held a fetus for more than 20 weeks is called a neonate. These terms are used to describe a woman's previous pregnancies in her history and to record medical information during pregnancy or other circumstances. In many medical and legal definitions, pregnancy is divided into three parts (trimester). There is the highest risk of miscarriage in the first trimester or first trimester. During the second or second trimester, fetal growth can be assessed, and from the third or second trimester, the fetus can survive outside the uterus. The first trimester of pregnancy refers to the period before the 12th week of pregnancy. Most abortions, embryonic development, and organ building also occurred during this period.

1-1-1 Division of pregnancy

There are generally two types of pregnancy divisions: 1 -Division of the first type. The first 3 months of pregnancy: from the first week to the end of the 14th week of pregnancy. The second trimester of pregnancy: from the 15th week to the end of the 28th week of pregnancy. Third trimester of pregnancy: from week 29 until delivery.

2 -Division of the second type First half of pregnancy: 0-20 weeks.

Second half of pregnancy: 20-40 weeks.

1-2 Improper pregnancy

Improper pregnancy remains one of the leading causes of maternal mortality. However, because today, using modern diagnostic methods, it is possible to detect most miscarriages early. Current treatments are more conservative than past treatments. The main focus has shifted from emergency surgery to control hazardous bleeding to medical treatments aimed at avoiding surgery and preserving the anatomy of the reproductive system and maintaining fertility.

1-3 Epidemiology of abnormal pregnancy

The incidence of miscarriage is 1.5-2% of all pregnancies. The rate of miscarriage is higher in blacks and other minorities than in whites in all age groups. This type of pregnancy progressively increases with age in all races and is 3-4 times more likely in women aged 44 to 35 than women aged 15 to 24. In nulliparous women, pregnancies that occur after at least one year without contraception are 2.6 times more likely to be tubular. Additional risks in infertile women are associated with specific therapies. These include reversal of sterilization, tuboplasty, ovulation induction, and IVF. The possibility of tubular implantation predisposes to the hormonal changes that characterize ovulation-stimulating cycles with clomiphene citrate and gonadotropins. About 1.6-1.4% of pregnancies with ovulation induction are abnormal types. In many of these patients, the result of hysterosalpingography is normal and there is no sign of intraoperative tubular pathology. Ovarian hyperstimulation with high estrogen concentrations may play a role in tubal pregnancy. Other predisposing factors include placement of the embryo in the upper part of the uterine cavity, fluid reflux into the fallopian tube, and predisposing tubular factors that prevent the refluxed embryo from returning to the uterine cavity (1).

1-4 Types of miscarriages

The most common types of miscarriages are tubal pregnancies and unusual types include heterotopic pregnancies, abdominal pregnancies, ovarian pregnancies, interstitial pregnancies, cervical pregnancies, and cesarean sections.

1-4-1 Tubular pregnancy

1-4-1-1 Etiology and risk factors

Damage to the fallopian tubes is caused by inflammation, infection, or surgery. Inflammation and infection can cause damage without completely

blocking the tube. Tubal obstruction may be due to salpingitis, incomplete tubal closure, tubal sterilization surgery, incomplete salpingectomy, or congenital atresia of the middle tube. Damage to the mucous membrane of the fallopian tube or fimbriae is responsible for about half of all tubular pregnancies. Tubular diverticula may lead to abnormalities that trap or obstruct the blastocyst. Tubal pregnancy may occur in a blocked tube if the opposite tube is open. In general, 70% of abnormal pregnancies are in the ampulla region, 12% in the ischemic region, 11% in the fimbriae, and 2% in the cornea. Independent risk factors that always indicate the risk of tubal pregnancies include:

1. Previous PID fixed by laparoscopy. 2. Previous tubal pregnancy.

3. Current use of the IUD. 4. Previous tube surgery to treat infertility. 5. Previous abdominal surgery. 6. Sterilization. 7. Diethyl acetylbestrol. 8. Smoking.

1-4-2 Heterotopic pregnancy

Simultaneous pregnancies in two different areas mean implantation. The most common combination is an intrauterine pregnancy and an ectopic pregnancy, most of which are in the fallopian tube.

1-4-3 Abdominal pregnancy

Implantation in the peritoneal cavity, which is referred to as abnormal abdominal pregnancies, is so rare that its estimated incidence is about one case per 10,000 pregnancies and one case per 100 abnormal pregnancies. In this type of pregnancy, implantation usually occurs in areas such as the omentum, the lateral wall of the pelvis, the broad ligament, the cochlea, the spleen, the intestine, the liver, the diaphragm, and the cervix.

1-4-4 Ovarian pregnancy

About 3% of all miscarriages are due to ovarian pregnancy. Symptoms of this type of pregnancy is very similar to the more common types of symptoms in tubal pregnancies. Of course, it should be noted that ovarian pregnancy has specific diagnostic criteria that are mostly academic, which includes the following materials. The ipsilateral tube is intact and is clearly separated from the ovary. Occupation of the ovarian position by the pregnancy sac.

Ovarian ligament attachment of the pregnancy sac to the uterus.

Existence of ovarian tissue in the wall of the pregnancy sac. Treatment in almost all cases is surgery.

1-4-5 Intermediate pregnancy

A maximum of about 2% of abnormal tubular pregnancies are implanted in an interstitial segment within 1-2 cm of the uterine wall. Conventional treatment for interstitial pregnancy has been hysterectomy or corneal resection through laparotomy.

1-4-6 Cervical pregnancy

Cervical pregnancy is a rare type of miscarriage which is occurred in implantation in the duct and cervix. It is somewhat more common in people who become pregnant with ART. The probability of this type of pregnancy was 1 in 1000 pregnancies resulting from IVF. Cervical pregnancy is associated with vaginal bleeding without a door (its classic sign), enlargement (dilation) of the large and soft cervix, and the appearance of bloody or cyanotic, soft and large (hourglass cervix). Common treatments for pregnancy include curettage and hysterectomy, which are used to control bleeding if necessary. But other treatments are performed with the aim of minimizing this risk such as cervical cerclage, intravascular injection of vasopressin, transvaginal ligation of the cervical branches of the uterine artery. Similar methods such as intracervical balloon tamponade and bilateral ligation of the uterine artery or internal iliac artery have been used to control postoperative bleeding.

1-4-7 Pregnancy in cesarean section scar

It accounts for approximately 6% of all miscarriages in women with a history of cesarean section. These abnormal pregnancies are thought to be caused by embryo migration from a defect in a cesarean section scar. Its clinical manifestations are highly variable, ranging from vaginal bleeding with or without pain, to uterine rupture and hemorrhagic shock. The best treatment for it has not yet been determined, and therefore several treatment options such as vaginal resection, laparotomy or laparoscopy, topical injection

of potassium chloride, or treatment with systemic or topical methotrexate are used.

1-5 Diagnosis of miscarriage

Diagnosis of miscarriage is associated with many complexities and difficulties due to the wide range of clinical manifestations. The diagnosis and management of a ruptured fallopian tube is very clear. The primary goal is to achieve homeostasis. If abnormal pregnancy can be detected before rupture or irreparable damage to the fallopian tube, then future fertility optimization can be considered. By taking a history and physical examination, patients at risk can be identified, and the likelihood of being diagnosed with a miscarriage before a rupture can be increased (2).

1-6 Methods of assisted reproduction (artificial insemination)

Assisted reproduction methods include all methods that use direct manipulation of oocytes outside the body.

1-6-1 Laboratory method

In vitro fertilization is the first and most common form of auxiliary fertilization today. In standard IVF, 50,000 to 10,000 sperm are placed in a culture medium with an oocyte to fertilize, and then the resulting embryo is transferred to the uterus.

In vitro fertilization, or IVF, is a method in which egg cells are fertilized with sperm in vitro and one or more egg cells are obtained after several stages of "8-cell" or "embryo 5" cell division. "Fasting" is placed in the uterus to allow the fetus to grow normally. In this method, ovulation is first induced in the female and after a suitable number of eggs are obtained, they are cultured in the laboratory. After the eggs mature, they fertilize the egg with the right sperm. There are different methods depending on how the sperm causes the egg to fertilize. For example, if a certain number of sperm are inoculated into a culture medium containing an egg so that sperm with the right motility, shape, and physiology can fertilize the egg, this is called IVF. However, if the appropriate sperm is selected and inoculated into the egg using a microinjection device, this method is called intracytoplasmic injection of sperm (3). Artificial insemination was first performed in the world in 1978 in the United Kingdom by Dr. Robert Edwards, who won the Nobel Prize in Physiology or Medicine in 2010. Louise Brown, the child born of this fertilization, was born on July 25, 1978. Since then, about 5 million children have been born this way in the world.

1-6-2 Cytoplasmic method sperm

Intracytoplasmic injection of sperm, which is widely used today, is performed using sperm isolated from ejaculate or sperms obtained by microsurgical aspiration of sperm from the epididymis or removal of sperm from the testis.

1-6-3 Transfer of sperm and oocyte tubes or transfer of gametes into the fallopian tube

Another ART procedure is gamete intubation, which was introduced in 1984 as a form of IVF and has been a successful alternative to infertile couples with unknown cause or cervical or immunological causes, mild endometriosis, or a few cases of male fertility.

GIFT Placing the sperm and egg combination directly into the fallopian tubes consists of 3 steps:

1. Ovarian stimulation and monitoring: The initial steps of the main ART processes are the same. First, ovarian stimulation is used to create several eggs to increase your chances of successful fertilization. During ovarian stimulation, the ovarian response to hormonal drugs is monitored and egg formation is assessed.

2. Ovulation: In GIFT, eggs are often taken laparoscopically and sperm are prepared in a manner similar to IVF. Oocytes are examined under a microscope to determine their maturity before combining with sperm and transfer to the fallopian tube.

3. Gamete transfer: As soon as the doctor announces that the eggs are ready to transfer, the sperm and egg are placed together in a special catheter. The doctor inserts this catheter with a laparoscope and injects the gametes directly into the fallopian tube.

Thus, the process of fertilization takes place in vivo or in a laboratory setting under normal conditions, like a fertile woman. The developing embryos remain in the fallopian tube and then move into the uterus like a normal pregnancy for implantation.

1-6-4 Transfer of eggs from fertilization of sperm and oocytes or zygote into the fallopian tube

Transfer of eggs from fertilized sperm and oocytes or zygotes into the fallopian tube is another type of assisted reproduction procedure that uses direct manipulation of the oocyte outside the body.

1-6-5 Transfer of multicellular embryo to fallopian tube

In the last three methods, simultaneous laparoscopy is required for transmission. Also, in all ART assisted reproduction methods, the following steps are performed jointly:

Controlled ovarian stimulation is performed using gonadotropins, which follicular growth is monitored by vaginal ultrasound and serum estradiol levels are monitored simultaneously.Prevent LH surge untimely and therefore premature ovulation .Establishment of the final stage of oocyte maturation by hCG injection . Obtaining oocytes.

Fertility of eggs obtained by IVF or ICSI method .In vitro growth and culture of embryos .Supporting the total phase or preparing the endometrium using exogenous progesterone .Transfer the embryo into the uterus and freeze the extra embryos.Assessment of fertility status in the first trimester of fertility.

1-6-6 Folliculogenesis

In an unstimulated cycle, a number of follicles (8 to 9 follicles) in the luteal phase begin their pre-growth cycle. About the middle of the follicular phase of the next cycle, one follicle emerges from them and as the growth of this follicle continues, the growth and development of other follicles in this selected cohort stops. Follicular growth in a non-stimulated cycle with hormonal feedback causes LH surge in the middle of the cycle, which plays a very important role in completing ovarian maturation and ovulation. Progesterone secretion begins before ovulation and increases markedly after ovulation. The secretion of estradiol in the follicular phase also causes the endometrial epithelium to grow and proliferate. The secretion and presence of progesterone is critical for the development of endometrial maturation and stroma to provide a suitable site for implantation in the middle of the secretory phase. If successful implantation occurs, stimulation of the hCG secreted on the corpus luteum will continue to secrete progesterone until the placenta can completely replace it at 8 to 10 weeks of gestation and the placenta will continue to secrete progesterone completely. It should be noted that the first IVF delivery was from an oocyte obtained during a normal, unstimulated menstrual cycle. IVF is still possible with a normal cycle and may also be performed in some elderly patients with low ovarian reserve who have previously failed ovulation-stimulating cycles or who are unable to stimulate ovulation due to complex medical conditions. However, in this method, the rate of non-completion of the cycle is high (25 to 75%, especially due to unplanned ovulation, which eliminates the chance of ovulation). Even if oocyte recycling and fertilization are successful, there will be only one embryo in natural cycles and, of course, there will be little chance of nesting. In these cycles, endogenous HL serum levels should be monitored frequently to prevent premature serum LH and premature release of oocytes. Ovulation is then performed 36 to 40 hours later. GnRH antagonists can also be used to prevent untimely serum LH.

1-6-7 Mild ovarian stimulation

Very mild stimulation with clomiphene citrate from Cycle Syndrome can be done for 5 to 8 days.

In this method, compared to the normal cycle, the rate of cycle cancellation is somewhat less and the number of oocytes obtained and embryos transferred and the rate of pregnancy is higher.

As with the normal cycle, GnRH antagonists are used to prevent untimely serum LH and hCG is used for the final maturation of oocytes. Alternatively, intermittent stimulation of clomiphene citrate and gonadotropin can be used (albeit in small amounts).

It has been shown that stimulation of follicular development is more successful than the use of clomiphene citrate alone. Intermittent use of clomiphene and exogenous gonadotropin in patients with a previous poor response to ovarian stimulation due to stimulation of the endothalamic-pituitary-ovarian endogenous system by clomiphene is a treatment protocol.

1-7 Abortion

Abortion before the start of the 22nd week of pregnancy is called an abortion. The most important sign of abortion is bleeding. If this happens after the first trimester of pregnancy, it is called a late abortion.

1-7-1 Definition of recurrent spontaneous abortions

The occurrence of at least three miscarriages in the first trimester of pregnancy is called recurrent miscarriage. It is defined as a miscarriage more than two or three times before 24 weeks of gestation. By most definitions, it is a fertility defect in 1-5% of patients who experience it. This defect is undoubtedly of multifactorial origin (4). The frequency of these abortions is said to depend on its definition, so its prevalence is estimated at 1-3%. In case of abortions that have been repeated twice without a previous successful pregnancy history. This rate increases. In general, 10-15% of pregnancies end in abortion, although this phenomenon is very rare, it is a very disappointing experience for patients and doctors because usually there is no definite reason and reliable treatment for such abortions. In general, abortion can be considered a natural way to select children with healthy genomes. In fact, after a study by Beau et al. And Hasold et al. On seminal fluid, it was accepted that 50% of abortions originated from chromosomal abnormalities. Also, cytogenetic studies of embryos created by in vitro fertilization (IVF) have shown that only 50% of seemingly normal embryos are chromosomally normal. The risk of developing fetal chromosomal abnormalities gradually decreases during pregnancy until it reaches 1% in newborns. Following this process (until fertility) reveals that most miscarriages occur in the very early stages of pregnancy.

1-7-2 Factors affecting recurrent miscarriage

One of the effective factors in abortion is the age of the mother. Aging reduces the function of the ovaries and the number of healthy eggs and the production of embryos with chromosomal defects (5). The indicator of this correlation is trisomy of chromosome 21 as well as the results of cytogenetic studies of embryos before transfer to the mother. Another factor is the results of previous pregnancies. The risk of miscarriage for young mothers who have no previous history but whose previous pregnancies have been successful is about 30 percent; if all previous pregnancies have failed, it will rise to 50 percent.

1-7-3 -Etiology of recurrent spontaneous abortion

In general, four reasons for spontaneous abortion are given:

1. (Infection (1%)

1. Chromosomal abnormalities (7-50%)

3. Hormonal abnormalities (5-20%)

4. Anatomical abnormalities (5-10%)

But overall, in 80% of cases, immunological abnormalities (autoimmune disorders) and other immunoassays (alloimmune disorders) are involved (6).

1-8 -Genetic factors

1-8-1 -Anoploidy

The most common chromosomal abnormalities are autosomal, polyploid and monosomal X chromosome trisomies. In most trisomies, the effect of maternal age is seen. Anoploids contain most of chromosomes 16 and 18. Triploidy and tetraploidy are the cause of 30% of spontaneous abortions due to chromosomal abnormalities.

Triploid embryos formed by double sperm fertilization usually have the genetic formula "XXX and 69" or "XXY and 69", and tetraploid embryos usually last less than 4 or 5 weeks. Monosomal chromosome (X) is the most common chromosomal abnormality, accounting for 15-20% of all miscarriages. In general, the frequency of chromosomal abnormalities is lower in women under 36 years of age.

1-8-1-1 -Trisomy 21

Down syndrome or trisomy 21 is the most common and well-known chromosomal disorder to date and is by far the most common genetic cause of moderate mental retardation. About 1 in every 800 babies born has Down syndrome, and the incidence is much higher

among live births of mothers aged 35 and older. Two notable features of the population distribution of this disease are noteworthy: increasing maternal age and its specific distribution within families. In 1959, it was discovered that people with Down syndrome have 47 chromosomes, and the extra member is a small acrocentric chromosome, hereinafter referred to as chromosome 21.

Down syndrome can be diagnosed at birth or shortly thereafter by dysmorphic features (varying among patients) that produce distinct phenotypes. In about 95% of all patients, Down syndrome is caused by trisomy on chromosome 21, which results from the meiosis of chromosome 21 pairs not being separated. As mentioned earlier, the risk of having a child with trisomy 21 increases with increasing maternal age, especially in those over 30 years of age. The meiotic error responsible for causing trisomy 21 usually occurs during our meiosis (about 90% of cases), and mostly in meiosis I, but about 10% of cases of the disease occur in paternal meiosis, usually in paternal meiosis II.

1-8-1-2 -Trisomy 18

Trisomy 18 features always include mental retardation and growth retardation and often include severe heart deformity. The incidence of this condition in live infants is about 1 in 7,500 births. The incidence of trisomy 18 is much higher at fertilization, but 95% of all pregnancy products with trisomy 18 miscarry spontaneously. Postnatal survival is also low, and survival of more than a few months is rare. At least 60% of patients are female, which is probably due to their preferential survival.

1-8-1-3 -Trisomy 13

The incidence of trisomy 13 is about 1 in every 15,000 to 20,000 births. Trisomy 13 is clinically severe, with about half of patients dying within the first month of life. Like many other trisomies, these patients are associated with the older age of the mother, and the extra chromosome is usually caused by the phenomenon of segregation in maternal meiosis I. Determining the karyotype of infected infants or fetuses is required for clinical confirmation. About 20% of cases result from an unbalanced displacement (7).

1-8-2 -Abnormalities in chromosome structure

The most common change in chromosome structure is displacement. Cytogenetic studies show that the frequency of this abnormality is 3-5% in sick couples and almost twice as high in women as in men. Abortion and fetal abnormalities depend on the size, location and type of change in chromosome structure. When a couple has these types of abnormalities, their fetus is 5 to 5 percent more likely to be infected (8).

1-8-3 -Multigenic factors

Single or multifactorial factors influencing the reproductive process (which are rarely detected) can cause miscarriage. For example, asymmetric deactivation of the X chromosome, which results from 90% deactivation of one parent-specific allele, is more common in mothers with spontaneous abortions.

1-8-4 -Anatomical abnormalities of the uterus

Undoubtedly, anatomical abnormalities of the uterus are one of the factors influencing abortion (in the first trimester). Evidence has been shown that surgery (to correct these disorders) is usually of little success.

1-8-5 -Environmental factors and life habits

Consumption of 5 or more units of alcohol per week and 375 mg or more of caffeine per day during pregnancy increases the rate of miscarriage. Also, there is a weak link between smoking and miscarriage. Heavy metals (such as lead and mercury), organic solvents, and radioactive ionizing radiation, which are considered environmental teratogens, increase the rate of miscarriage. Couples' jobs, environmental pollution, and smoking can reduce sperm quality and cause miscarriage. Couples' jobs, environmental pollution, and smoking can reduce sperm quality and miscarriage early in pregnancy (9).

1-8-6 -Hormonal abnormalities

Maternal hormonal disorders (diabetes and thyroid failure) are effective in causing miscarriage. High maternal hemoglobin levels in the first trimester of pregnancy increase the risk of miscarriage (10). Controlled diabetes does not increase the risk of miscarriage (11), but thyroid disorders increase the risk of miscarriage (12). Reports of progesterone deficiency

have been implicated in abortion. Some researchers believe that progesterone deficiency may, in some cases - not always - be found to be beneficial.

In human placental gonadotropin deficiency, administration of this substance is not always beneficial(13).

Polycystic ovary syndrome (PCOS) is associated with miscarriage. The hallmark of this disease is excessive secretion of LH hormone, which is considered as one of the causes of abortion, but reducing LH secretion (by inhibiting the pituitary gland) has little effect on preventing abortion (8)).

In 25-30% of women with RSA, there is a history of delay of more than 12 months before pregnancy, the most common cause of which is an abnormality in the ovary (10).

Hyperprolactinemia has been reported in some sources as a factor in increasing abortion, but there are insufficient reasons for this (14).

1-8-7 -Immunological abnormalities

When ionological abnormalities cause abortion, the probability of a successful pregnancy of the mother from three abortions is 30%; this probability decreases to 25% after 4 miscarriages and to 5% after 5 miscarriages (15) (Figure 1).

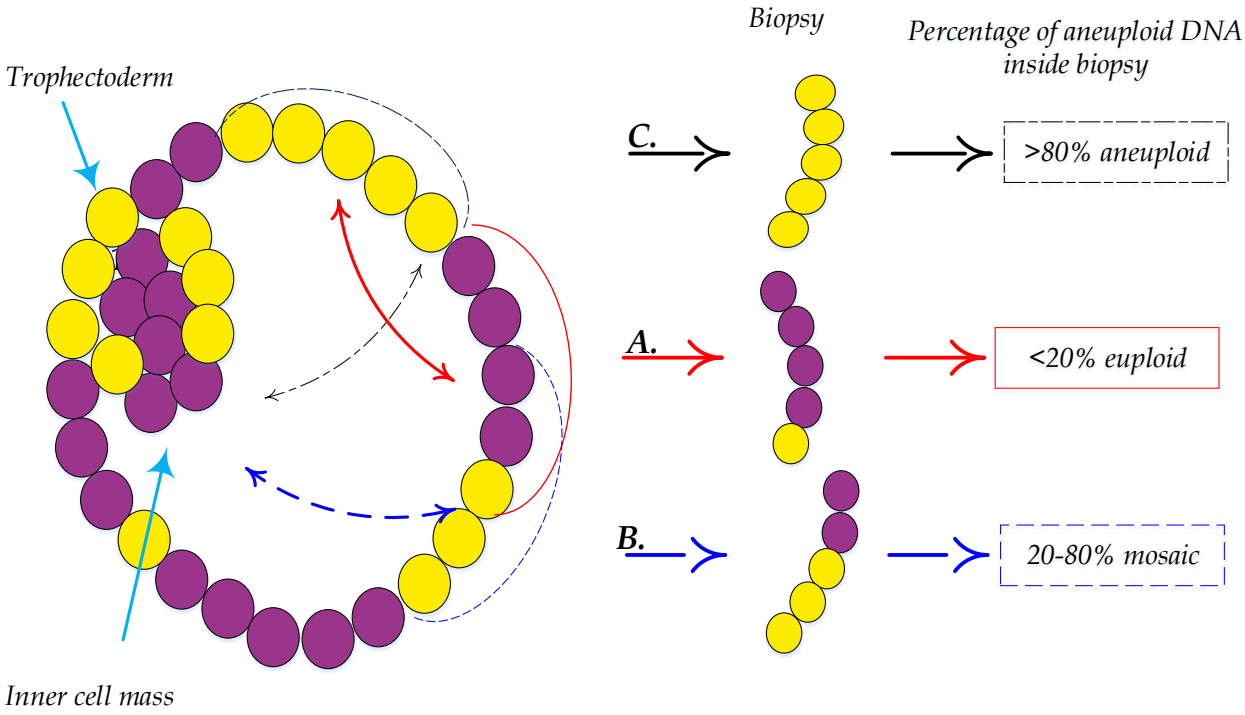

Figure 1 . Percentage of aneuploidy DNA inside biopsy for diagnosis.

1-9 -Polymerase chain reaction

Polymerase chain reaction is a laboratory technique that allows the amplification of a specific piece of DNA that exists between two known sequences. DNA amplification occurs after the primer is attached to the end of specific sequences of the template DNA. The polymerase chain reaction technique was developed in 1985 to determine the sequence of DNA strands by Kerry Mollis. Kerry Mollis was awarded the Nobel Prize in 1993 for his invention of PCR (Figure 2).

In this technique, DNA complement strands are synthesized using dNTPs in the presence of the polymerase enzyme. The resulting two strands of DNA can be separated by heat, and then the temperature must be adjusted to allow the primers to adhere. DNA elongation is performed using DNA polymerase at the optimum temperature for enzyme activity. Repeating the steps of opening two DNA strands, attaching primers, and lengthening is called a PCR cycle. Each newly made DNA strand is used in the next cycle as a new pattern strand, and the target DNA fragment is

made from it. The first cycle of PCR is performed on the initial pattern and will continue as long as the polymerase enzyme is active or until the start of the next cycle. In the second PCR cycle, the strands are made of a certain length that is limited to two primers. From the fourth cycle onwards, the DNA sequence is amplified exponentially, so the number of final copies of the target sequence is defined by the formula (2n-2n) X. In this formula, n is the number of bicycles and 2n is the initial product of the first and second cycles, which have certain lengths, and X is the number of copies of the original pattern. If we assume that the work of this PCR is 100%, the DNA of the template will be multiplied 220 times. It should be noted that the performance of PCR varies depending on the type of template DNA and the optimization conditions. The target sequence in PCR also includes two-end primers. Exponential amplification of target DNA does not occur indefinitely, and factors prevent maximizing efficiency in each cycle. The effect of these factors is greater in the end cycles. For example, the Baghdad DNA sequence is amplified 106 times from 25-30 cycles of PCR, and the ratio of enzyme to DNA decreases due to the increase in the DNA molarity of the template. The activity of enzymes is also reduced by thermal decomposition. On the other hand, the high concentration of pattern filaments causes the filaments to stick together and compete with the primer adhesion.

Two factors have contributed to the development of PCR:

A) The use of DNA polymerase stable enzymes at high temperatures

B) The use of devices that automatically generate temperature cycles.

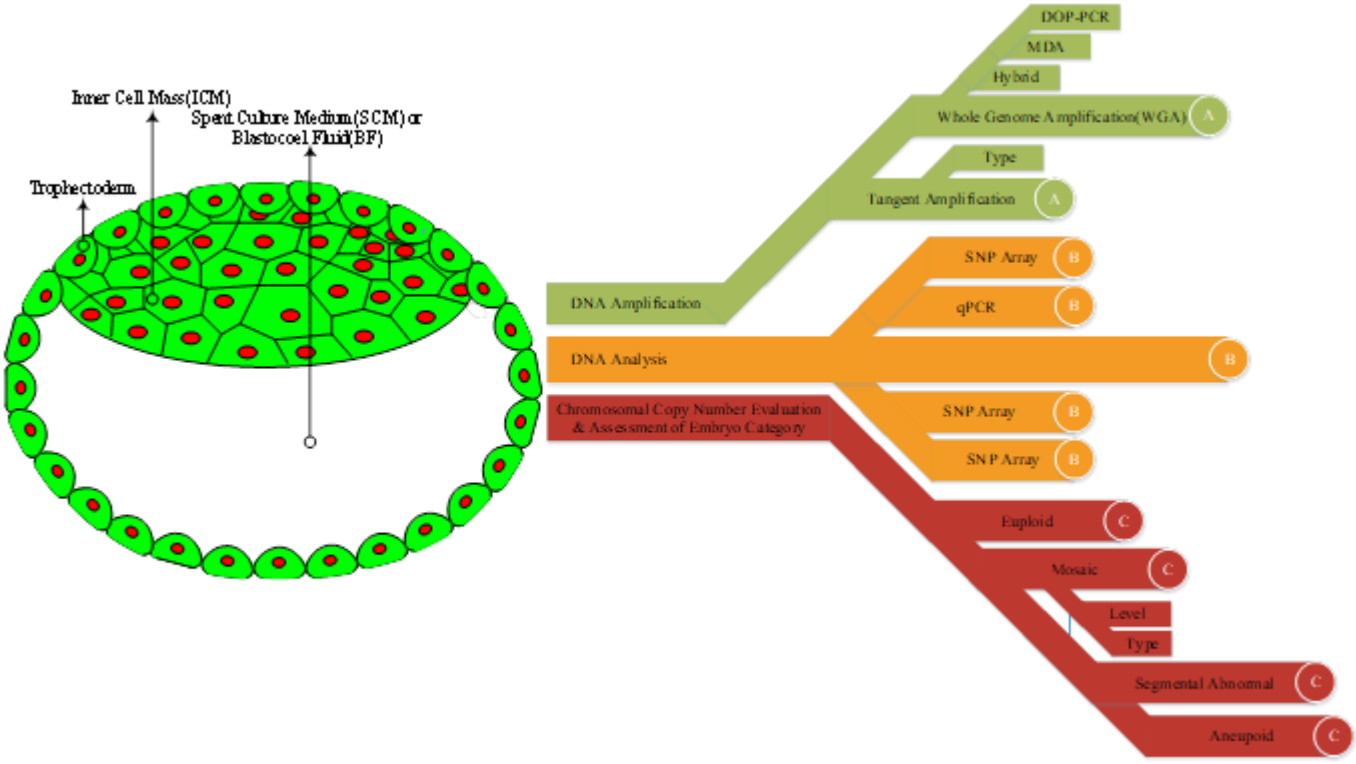

Figure 2. DNA amplification, DNA analysis alongside with chromosomal copy number evaluation and assessment of embryo category in some types of PCR for diagnosis.

1-9-1 -Factors affecting the success of PCR

1-9-1-1- Incubation of two strands of DNA

Separation of two DNA strands takes place at a temperature of 92-100 ° C. Rising temperatures cause DNA damage. This affects the reproducibility of the results. Therefore, high temperatures should be avoided. PCR is typically used to separate two strands of DNA at temperatures between 95-92 ° C, but the nucleation temperature must be based on different DNA patterns (if the target DNA is in the

heterochromatin region to separate DNA strands require higher temperatures).

1-9-1-2 -Sticking primers

Calculating the adhesion temperature of primers is a starting point and an important factor in PCR optimization. If a single strand of DNA is slowly cooled, the strands that complement each other in terms of the play sequence are connected in a specific way. In the PCR process, after priming the template DNA, each primer must be optimized practically by repeating the experiments.

1-9-1-3 -Elongation of DNA strand

Supplemental strand elongation begins with 'OH3' at 72 ° C, which is the best temperature for Taq DNA polymerase. The development of new strands depends on the activity of the DNA polymerase enzyme. This enzyme was first extracted from the bacterium Termus aquaticus belonging to the hot spring in Yellowstone, which simplified and automated PCR. This enzyme is most active at 72 ° C.

If the amplified DNA fragment is large, the amplification time can be increased. But in most cases, two minutes is enough. Factors such as divalent cations (such as Mg2 +) are very effective in the activity of this enzyme. The PCR process creates a new copy of each DNA strand molecule, which is actually the target region. Each new version can be rewritten and produced in a similar cycle.

1-9-1-4 -Number of cycles

The number of cycles is usually considered to be between 25-35 and increases with. Also, due to the decrease in enzyme activity, the number of cycles is not selected more than 40 cycles.

1-9-2 -Types of PCR techniques

1-9-2-1 -ARMS technique

The shaky mutation amplification system, or ARMS, is a simple and rapid way to detect point mutations, restricted fragment length polymorphisms (RFLPs), small deletions or additions in the DNA molecule sequence. In this method, the reaction is performed in two separate tubes, one of which contains mutated type primers and the other contains normal type primers. If amplification occurs in a tube containing a mutated primer, mutation has occurred in the target DNA, and amplification in a tube containing a normal primer indicates that no mutation has occurred (16).

1-9-2-2 -Nested-PCR technique

In this method, two pairs of primers are used to increase the sensitivity of PCR. First, with a pair of first primers over 15-30 cycles, specific pieces of target DNA are amplified. Then the resulting PCR product is transferred to another tube and used as a template and is performed by the second pair of primers in the second stage of PCR (17).

1-9-2-3 -RT-PCR technique

The primary pattern in RT-PCR is a single-stranded RNA molecule. Since DNA polymerase is not able to use RNA as a template, another step has been added to PCR. During this stage, using the reverse transcriptase enzyme, from the RNA pattern, its complement CDNA is synthesized and amplified by PCR technique (18).

1-9-2-4 -Multiplex-PCR technique

In this method, several pairs of specific primers are used for different purposes. In clinical microbiology, using this method, it is possible to identify several disease agents in a sample at the same time and to diagnose mixed infections. It is a type of PCR reaction in which two or more sites of the target sequence are amplified simultaneously in a PCR reaction. Separate primers are designed for each target DNA fragment. The PCR product contains a mixture of parts of different lengths that can be differentiated by electrophoresis on agarose gel (19).

1-9-2-5 -QF-PCR technique

Quantitative fluorescent polymerase chain reaction has been used in diagnostics in the UK for over 10 years and has proven to be a fast, cost-effective, robust and accurate prenatal test for common aneuploidies. Its specific benefits include the diagnosis of triploidy, mosaicism of the stem cell infection.

QF-PCR can be used to detect copying of chromosomal numbers by incremental duplicate sequences at polymorphic sites. These duplicate sequences are

amplified by PCR and the labeled products are separated by gel electrophoresis.

QF-PCR was introduced to the UK National Health Service as a valid diagnostic test in 2000 (20) and then to other UK genetic centers (21) and privately in the UK and Europe. Introduced (21). Primer pairs for polymorphic loci (markers) together led to a rapid, efficient, and inexpensive diagnostic test for trisomy 13, 18, and 21 aneuploidies and sex and triploid chromosomes, which are now available in commercial kits from a number of different companies. Sometimes it is not possible to spend this time for various reasons, such as the anxiety of parents, especially the pregnant mother, or the lack of time to receive results before the end of the legal opportunity to terminate the pregnancy. If one of the families wants to get results faster due to high anxiety and also the risk that threatens the pregnancy, one of the most common chromosomal abnormalities - including aneuploidy (number abnormalities) of chromosomes 13, 18, 21, X or Y - has been specifically identified. QF-PCR technique can be used to accelerate the presentation of results (Figure 3). In this case, the anxiety caused by long-term waiting will be reduced. Due to the high sensitivity and specificity of this test and its relative equality of accuracy with chromosomal culture for the diagnosis of common anoploids, the answer to the family is provided with high confidence.

Also, in cases where the result of screening or ultrasound clearly raises the risk of a number of abnormalities on one of these chromosomes and the gestational age is more than 17 weeks, there is enough time to obtain a termination permit after the chromosomal culture results are ready. will not be. It should be noted that the termination permit of the infected fetus is issued only until the end of the 18th week of pregnancy (18 weeks and 6 days). In such cases, the QF-PCR technique will be helpful.

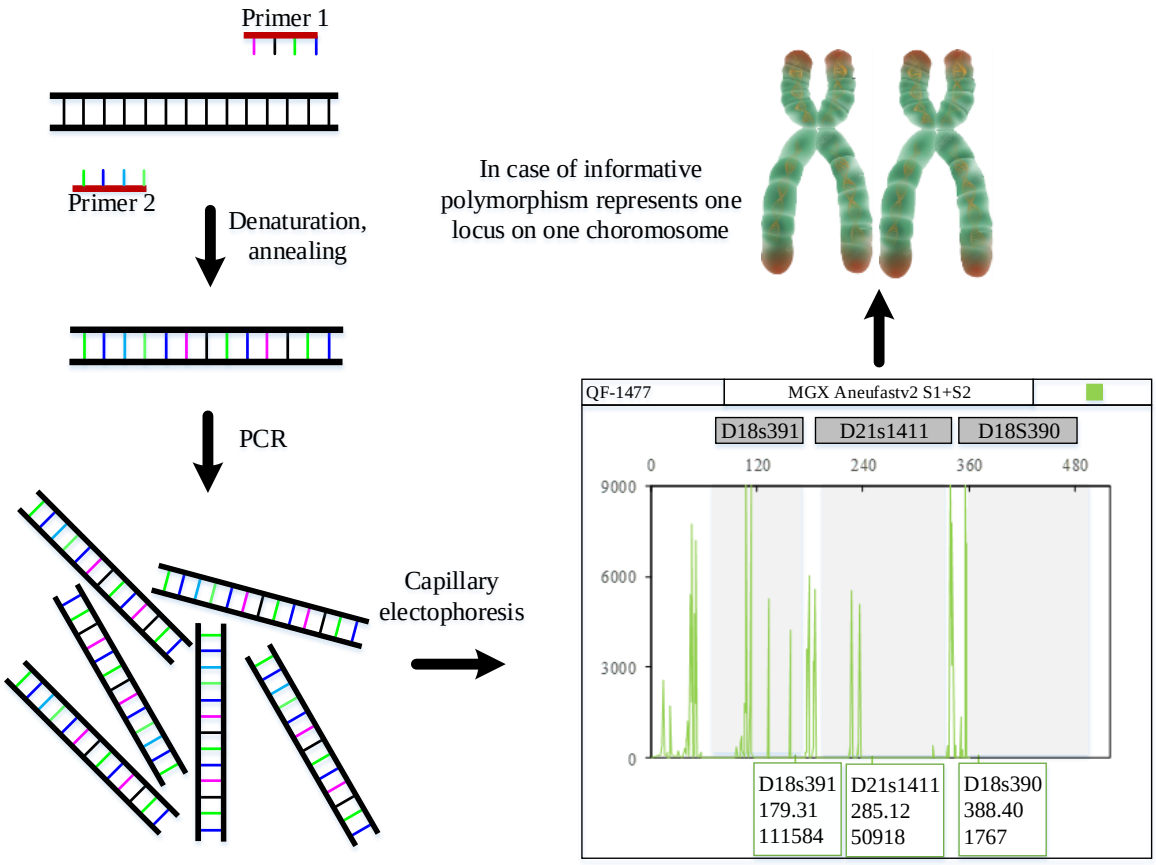

Figure 3. Position and arrangement primer, denaturation, annealing duration PCR function.

1-9-2-5-1 -QF-PCR as an independent test

Performing QF-PCR as a rapid initial test for aneuploidies raises questions about the benefits of this program with complete karyotype analysis that shows no signs of non-trisomy disorders. This was first possible in 2001 (22) Has been proposed and has since been widely considered with a number of retrospective reviews published. (23); (24). These reviews generally organized the karyotype test results form for prenatal samples and the number and nature of abnormal results that could not be detected by QF-PCR alone. Overall, this review suggests that the clinical significance of the prevalence of non-trisomic chromosome disorders in women at risk of trisomy is about 0.07%, close to the prevalence in the general population (25, 26).

However, with the higher resolution testing currently available, the prevalence of detectable non-trisomic disorders will be significantly higher in the general population.

Since then, two models for performing QF-PCR have been introduced as an independent test.

Model 1: Implemented by the Karolinska Institute in Stockholm, which gives women who were not at increased risk for non-trisomic chromosomal abnormalities the right to choose a rapid test for trisomies or a complete chromosome analysis, but not both.

Model 2: Includes QF-PCR testing of whole pregnancy specimens, regardless of referral, but sets low criteria for complete karyotyping of a subset of specimens. Introduced in the UK, the model is funded by the London Commission. With the imminent introduction of highly sensitive non-invasive screening for Down syndrome, the number of women who will have invasive testing will be reduced. For those at high risk for Down syndrome, invasive testing to confirm screening results will be a pre-term requirement, and QF-PCR should be considered as the method of choice for this rapid confirmation. Women at low risk for noninvasive screening will be identified later for invasive testing for fetal abnormalities in ultrasound screening.

For these women, an initial QF-PCR test, in the absence of a trisomy, must first perform a very expensive whole genome test, whether G-chromosome analysis or comparative genome array hybridization.

Therefore, QF-PCR will continue to play a key role in prenatal diagnosis in the future.

Conclusion

The benefits of QF-PCR are greater than other rapid aneuploidy diagnostic approaches. An important point is the significant difference in strategy and performance between other molecularly based methods for their inability to detect triploids. For triploid specimens, chromosome comparison analysis by MLPA, BAC, and comparison of genomic array hybrids may result in a natural diploid or MCC. Another important clinical advantage of QF-PCR is the ability to identify other cell lines at 10% MCC levels in female embryo samples and 20% (mosaicism) (26). It has been proven that despite all types of samples, this service is an affordable service and can receive results 6 hours after receiving the sample.

Author contribution

KB, MA, AAS wrote the manuscript, revised and conducted this study. All authors read the final edited version of the manuscript.

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Ethical Considerations

There are no ethical problems for this present review article.

Conflict of interest

The authors declare no potential conflicts of interest.

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