ejpmr, 2021,8(8), 86-95.

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

CORRELATION OF ABERRANT IMMUNOHISTOCHEMICAL EXPRESSION OF ABH ANTIGENS WITH POOR PROGNOSTIC FACTORS IN BREAST CARCINOMA

Anass Jarmoumi[1,2*], Soukaina Zouine[1,2], Salma Hasnane[1,2], Farida Marnissi[3] and Norddine Habti[1,2]

[1]Laboratory of Hematology, Cellular and Genetic Engineering, Faculty of Medicine and Pharmacy Casablanca, Hassan II University of Casablanca, Casablanca, Morocco.

[2]Laboratory of Cellular and Molecular Inflammatory, Degenerative and oncologic Pathophysiology, Faculty of Medicine and Pharmacy Casablanca, Hassan II University of Casablanca, Casablanca, Morocco.

[3]Pathology Department, University Hospital Ibn Rochd Casablanca, Casablanca, Morocco.

***Corresponding Author: Anass Jarmoumi**

Laboratory of Hematology, Cellular and Genetic Engineering, Faculty of Medicine and Pharmacy Casablanca, Hassan II University of Casablanca, Casablanca, Morocco.

Article Received on 09/06/2021 **Article Revised on 30/06/2021** **Article Accepted on 20/07/2021**

ABSTRACT

Studies of the expression of ABH antigens in different tissues have shown altered glycosylations related to the tumor process, inducing aberrant expressions correlated with advanced cancer stage and poor prognosis. Our main objective is to study experimentally the expression of ABH antigens on breast tumor tissues in a large sample of patients. This are an experimental study carried out on breast tumor tissue samples, from patients with blood group B. To further understand the modalities of ABH antigen expression in breast tumor tissue, and their association with breast cancer prognostic factors, an immunohistochemical study was performed on surgical specimens of breast tumor tissue, to verify the expression of HER2 receptors, B and H antigens. The study concerned 97 cases. Analysis of immunohistochemistry results of tumor tissues showed a loss of B-antigen expression in 83.5% of cases, of which 54.32% expressed H antigen. Loss of B-antigen expression is more observed in SBR III tumors with a percentage of 95.75%. The cases expressing the H-antigen are all SBR grade III. The patients presenting the profile antigen-B negative, and antigen-H positive have 91.2% of negative estrogen receptors. Estrogen receptors are negative in 78.9% of the B antigen negative patients. 86% of the tumor tissue of the B antigen negative patients is HER2 positive. The difference is significant ($p<0,05$) with patients who have maintained B expression and who are all HER2 negative.

Aberrant expression, is related to the initial oncogenic transformation and may precede the specific symptomatology of malignant conversion.

KEYWORDS: Antigens; Blood; Breast; Carcinoma; Histocompatibility.

1. INTRODUCTION

Breast cancer is the second most common malignancy in the world, and the most common in women. It is diagnosed in 1.7 million new cases each year, causing approximately 400,000 deaths per year worldwide.[1]

Breast cancer is a malignant proliferative process in the breast, of multifactorial origin, with an evolution that is difficult to evaluate and dependent on several genetic, epigenetic and environmental causes. Its prognosis is estimated by analyzing several clinical, histological and biological factors such as: histological grade, hormone receptors and HER2 oncoprotein overexpression.[2],[3]

Like all cancers, breast cancer is associated with glycosylation alterations affecting glycoproteins and glycolipids. Aberrant expression of glycan structures and the appearance of truncated structures, precursors or novel glycan structures may affect ligand-receptor interactions and thus interfere with the regulation of cell adhesion, migration and proliferation. Some unique alterations in tumor-associated glycosylation may be a distinct feature of cancer cells and, therefore, provide new diagnostic and even therapeutic targets. Aberrant glycosylation also alters ABH antigens of the ABO blood group system, which are also implicated in different types of cancers.[4],[5]

ABH antigens are complex structures of carbohydrates, covalently bound to glycoproteins or glycosphingolipids. They are present on several tissues in the human body, hence their important role in cell recognition, proliferation, adhesion and motility.[6]

Expression studies of ABH antigens in different tissues have shown altered glycosylations related to the tumor process, inducing aberrant expressions. These aberrations are manifested by loss of expression, onco-fetal expression and incompatible expression of another antigen, different from the one defining the subject

studied. Altered expression of ABH antigens on tumor tissues has often been correlated with advanced cancer stage and poor prognosis.[7], [8]

However, these findings are in contradiction with those of other studies performed in several populations and concerning several types of cancer. One of the most likely explanations for the controversy is the genetic and phenotypic polymorphism, especially ABO, which is ethno-dependent.

Thus, in recent years, several studies have concerned the involvement of ABH antigens in tumor progression, in different populations. In Morocco, in 2016, our team published preliminary results of the study of ABH antigen expression in tumor tissues collected from women with breast cancer. Some of the data remained incomplete and therefore did not allow to conclude in a significant and statistically valid way.[9] Thus, it has been reported that.

- Loss of B antigen expression on breast tumor tissue was observed in 96.3% of blood group B patients.
- Blood type B patients expressing H antigen on tumor tissue after loss of B antigen showed overexpression of HER2 receptor in 64.29%.

However, the sample size of blood group B patients with all data, including HER2, was small. To complement these results, our main objective is to study experimentally the expression of ABH antigens on breast tumor tissue in a large sample of blood group B patients. The ABH and HER2 immunohistochemical data are then correlated to some prognostic factors and to different anatomopathological and clinical features.

An approval from the Ethics Committee for Biomedical Research of Rabat (CERBR) - Faculty of Sciences Rabat, University Mohammed V, was granted for the realization of this study.

1. MATERIALS AND METHODS

This retrospective, cross-sectional, single-center, experimental study was performed on 97 tumor breast tissue samples obtained by different surgical techniques, in patients with blood group B (information taken from blood grouping cards) and operated for breast cancer.

After the selection of the patients' files at the Gynaecology Department of the Mohammed VI Centre, we selected the pathological anatomy reports of these patients and their specimens at the Pathological Anatomy Department of the UHC Ibn Rochd, respecting these inclusion criteria.

- The availability of the patient's initials;
- The accuracy of the hospitalization number on the medical record and the report;
- The surgical procedure was performed in the gynecology department of the Mohammed VI Center;
- The presence of malignancy is affirmed on the last report;
- The availability of representative samples of breast tumor tissue.

To further understand the modalities of ABH antigen expression in breast tumor tissue, and their association with breast cancer prognostic factors, an immunohistochemical study was performed on surgical specimens of breast tumor tissue, using murine anti-B antibodies (clone: 9621A8; DIAGAST, France) and anti-H (DiaClon Anti-H, clone: H-86/50, BIO-RAD, Switzerland), which were diluted to the appropriate concentrations, for maximum production of endothelial and epithelial cell staining.

Previously, anti-B and anti-H antibodies were checked with blood group B and O red blood cells before being used in immunohistochemistry. Red blood cells of each blood group were washed 3 times with 200µL of 0.9% isotonic NaCl solution and centrifuged at 1300 xg for 3 minutes. Then, a 2% red cell suspension was made by adding 1 mL of the isotonic solution. Reactivity was tested by adding 30µL of the antibody to 30µL of the corresponding red cell suspension. After incubation at room temperature for 15 minutes, the mixture was centrifuged at 145 xg for 3 minutes. Agglutination was read by eye. Reading and interpretation were performed according to the International Agglutination Score Code standards.

HER2 receptor status was not established in 37 patients. An immunohistochemical study was performed, using the rabbit anti-human HER2 antibody (from the kit: HercepTestTM, Code K5204, Dako, Denmark) according to the supplier's instructions.

Immunohistochemistry is a combination of immunological and histochemical techniques that allow the detection of intra-tissue expression of an antigen by a primary antibody specifically directed against that antigen. The primary antibody is recognized by secondary antibodies (universal antibodies) conjugated with fluorescent markers or with revealing enzymes (peroxidase, alkaline phosphatase) capable of transforming a colorless chromogenic substrate (e.g. Amino-3-Ethyl-9-Carbazole) into a colored product visible under the optical microscope. For the immunohistochemical study, we used a HercepTestTM immunostaining detection kit, K5204 Dako.[10]

All breast tissue samples collected for the immunohistochemical study were formalin-fixed and paraffin-embedded and were prepared in several steps. Noting that formalin fixation of the tissue generates intermolecular bridges between the macromolecules of the cell, which may result in masking of HER2 receptors and lack of antigen reactivity. Therefore, to demonstrate this receptor, tissue sections should be treated with an epitope restoration solution consisting of 0.1 mol/L

citrate buffer at a temperature of 98˚C for 15 minutes and then cooled to room temperature for 10 minutes.

After preparation of the sections by removing excess kerosene, and delineation of their contours with a Pap pen, we inhibited endogenous peroxidase activity by adding 3% hydrogen peroxide reagent for 10 minutes. Primary antibodies were applied for 60 minutes with a visualization reagent for HER2 receptors consisting of horseradish peroxidase-conjugated dextran polymer and goat anti-rabbit immunoglobin, and with another reagent for the visualization of ABO system antigens, consisting of dextran coupled with peroxidase molecules and goat secondary antibody molecules directed against mouse and rabbit immunoglobulins (EnVisionTM, Flex, Code: K8000, Dako, Denmark).

The sections were covered for 3 minutes with an extemporaneously prepared chromogenic solution, amino-3-ethyl-9-carbazole, which reveals peroxidase activity. Then, the slides were rinsed several times with distilled water before being incubated with Mayer's Hemalum for 5 minutes, and rinsed with running water.

Finally, the slides were dehydrated in 3 ethanol baths (3x5 minutes), thinned in 3 toluene baths (3x5 minutes) and mounted under coverslips with Eukitt sizing solution.

Read under the light microscope, the positivity consists of a brownish and granular staining at the cytoplasmic or membrane level.

Labeling with anti-B and anti-H antibodies was expressed as a percentage of labeled cells. Labeling was considered positive when the percentage of labeled cells was greater than 5%.

The HER2 score was determined according to the intensity of the labeling and the percentage of labeled cells.

To investigate the relationships between loss of B-antigen expression and H-antigen expression, the pathological features of each specimen and patient-specific prognostic factors, a statistical analysis of the results obtained was performed by the Chi-square test using the Statistical Package for the Social Sciences (SPSS), version 22.0. For all statistical tests used in this research, a two-tailed value of $p < 0.05$ was considered significant.

2. RESULTS

The study involved 97 cases of women with blood group B, meeting the inclusion criteria. The malignancy of the tumor was confirmed in the last pathology report. Representative samples of tumor tissue were available at the Laboratory of Pathological Anatomy of CHU Ibn Rochd.

Table 1 presents the clinical, histological and anatomopathological characteristics of the study population. Data on unavailable characteristics marked "not specified" were included in the first censuses. These cases were not considered in the statistical association analyses.

Before performing the immunohistochemical study, only 47 patients had all clinical, pathologic, and prognostic data included in our study. We expanded the sample size to 97, with a number of 50 patients. Of these, 37 were studied for additional HER2 immunohistochemical expression.

Table 1: Clinical and pathological characteristics of Patients with blood type B (n=97).

Characteristics	Patients with blood type B
Number of Patients	97
Average age (years)	51.27 [19-90]
Histological type	
Non-specific infiltrating carcinoma	70 (72.2 %)
Invasive lobular carcinoma	6 (6.2 %)
Ductal carcinoma in situ	6 (6.2 %)
Carcinoma with medullary aspect	3 (3.1 %)
Mucinous carcinoma	3 (3.1 %)
Invasive micropapillary carcinoma	2 (2.1 %)
Metaplastic carcinoma of non-specific type	2 (2.1 %)
Lobular carcinoma in situ	2 (2.1 %)
Invasive papillary carcinoma	2 (2.1 %)
Carcinoma with apocrine differentiation	1 (1.0 %)
Tumor size	
Average tumor size (cm)	4.27 [0.6-20.0]
T1: ≤2 cm	21 (21.6 %)
T2: 2 cm <t<5 cm	44 (45.4 %)
T3: ≥5 cm	32 (33 %)
Histo-pronostic grade	

SBR I	12 (12.4 %)
SBR II	38 (39.2 %)
SBR III	47 (48.5 %)
Estrogen receptor status	
Positive	29 (29.9 %)
Negative	58 (59.8 %)
Not specified	10 (10.3 %)
Status of progesterone receptors	
Positive	36 (37.1 %)
Negative	51 (52.6 %)
Not specified	10 (10.3 %)
HER 2 status	
Positive	43 (44.3 %)
Negative	48 (49.5 %)
Doubtful	6 (6.2 %)
Status of lymph node metastases	
Positive	50 (51.5 %)
Negative	28 (28.9 %)
Not specified	19 (19.6 %)
Status of vascular emboli	
Presence	39 (40.2 %)
Absence	56 (57.7 %)
Not specified	2 (2.1 %)

The results of the immunohistochemical study by anti-HER2 antibody showed receptor overexpression in 47.25% (43/91) of the tumors studied. Six cases showed HER2 membrane receptor labeling at equivocal score 2+.

Figure 1 shows examples of immunohistochemical labeling with the anti-HER2 antibody obtained in blood group B patients.

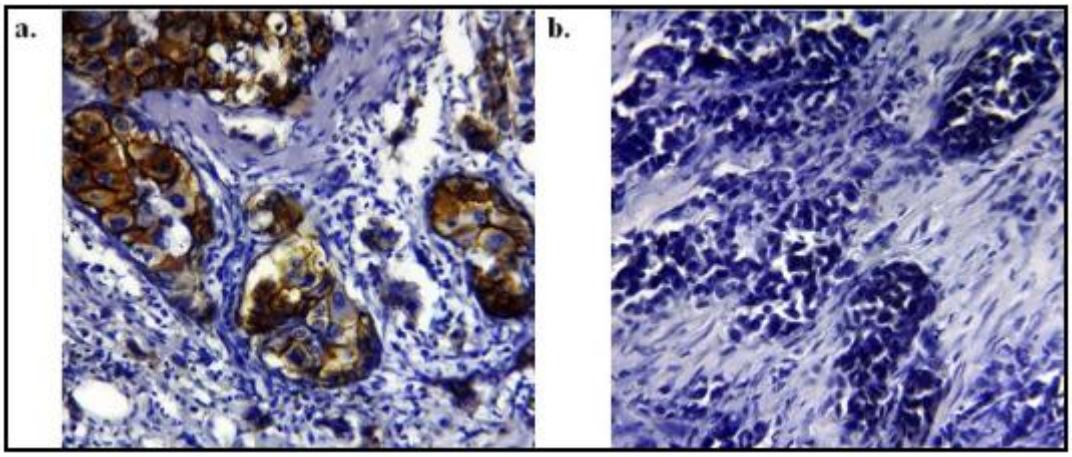

Figure 1: Examples of immunohistochemical labeling with anti-HER2 antibody on breast tumor tissue.

a*-Intense membrane HER2 overexpression on invasive tumor cell clusters (score 3+);*
b*-Absence of HER2 overexpression on invasive tumor cells (score 0+).*

Immunohistochemical expression of B and H antigens was revealed on tumor cells using anti-B and anti-H antibodies. Labeling was cytoplasmic or membrane-based. Table 2 summarizes the immunohistochemical profiles and results obtained with the 97 specimens.

Table 2: Expression profiles of B and H antigens in breast tumor tissue of patients with blood group B (n=97).

	Antigen B	Antigen H	Number of patients	Percentage (%)
Profile1	+	-	16	16.5 %
Profile 2	-	-	37	38.1 %
Profile 3	-	+	44	45.4 %
+ : positive - : négative				

Figure 2 shows examples of immunohistochemical labeling with anti-B and anti-H antibodies obtained in blood group B patients.

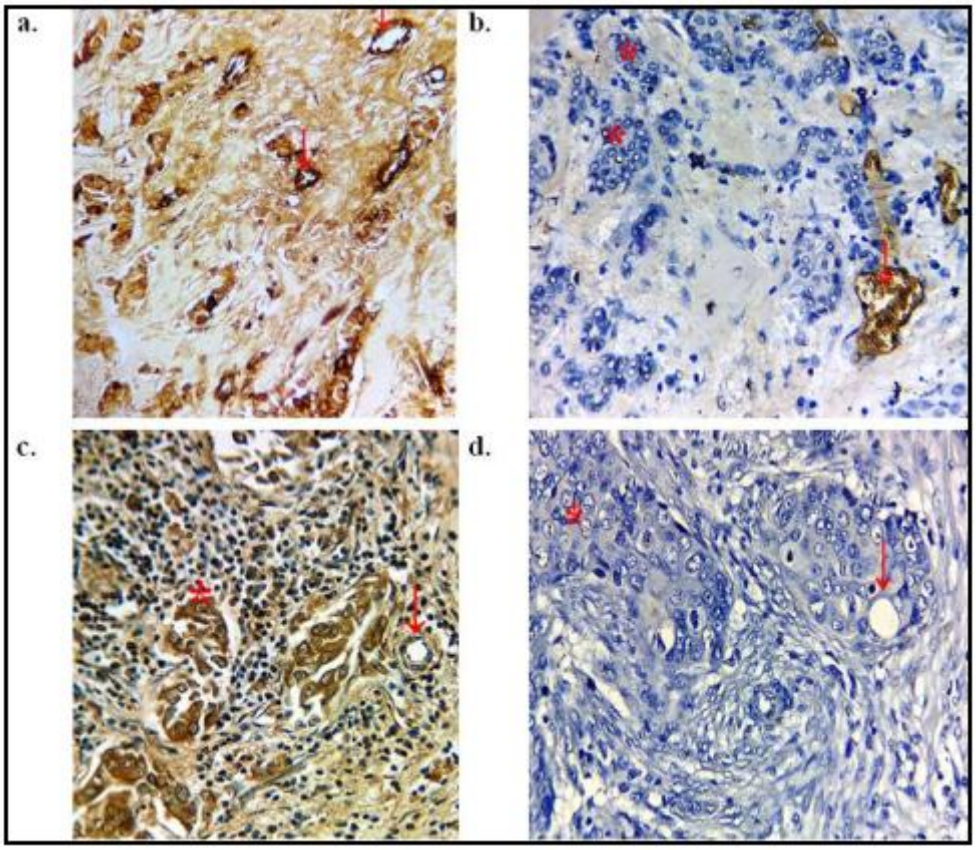

Figure 2: Examples of immunohistochemical labeling with anti-B and anti-H antibodies, on breast tumor tissues of blood group B patients.

a*-Immunohistochemistry with anti-B antibody showing intense and diffuse cytoplasmic and membrane expression by tumor cells, with intense expression on the internal control represented by vessels (↓);* ***b****-Immunohistochemistry with anti-B antibody showing an absence of expression of this marker at the level of tumor cells (*), with a positive internal control (vessel cells and red blood cells ↓) ;* ***c****-Immunohistochemistry with anti-H antibody showing intense cytoplasmic expression by tumor cells (*), with negative expression on vessels (↓);* ***d****-Immunohistochemistry with anti-H antibody showing absence of expression by tumor cells (*), as well as absence of expression at the level of vessels (↓).*

Analysis of the immunohistochemistry results of the tumor tissues showed a loss of B antigen expression in 83.5% (81/97) of the cases (profile 2 and 3), of which 54.32% (44/81) expressed H antigen (profile 3).

The bivariate statistical analysis involved the three B and H antigen expression profiles and several prognostic factors. A summary of the results of the analysis is presented in Table 3.

Table 3: Summary of associations between B and H antigen expression and different prognostic factors.

Characteristics	**Expression profiles of B and H antigens**			**p-value**
	Profile 1: B: Positive H: Negative	**Profile 2:** B: Negative H: Negative	**Profile 3:** B: Negative H: Positive	
Number of Patients	16 (16.5 %)	37 (38.1 %)	44 (45.4 %)	
Age (year)				
≤ 35 year	2 (12.5 %)	5 (13.5 %)	2 (4.5 %)	>0.05
35 – 70 year	13 (81.3 %)	32 (86.5 %)	37 (84.1 %)	
≥ 70 year	1 (6.3 %)	0 (0%)	5 (11.4 %)	
Histological type				
Non-specific infiltrating carcinoma	12 (75 %)	26 (70.3 %)	32 (72.7 %)	>0.05
Invasive lobular carcinoma	0 (0 %)	2 (5.4 %)	4 (9.1 %)	

Ductal carcinoma in situ	1 (6.3 %)	2 (5.4 %)	3 (6.8 %)	
Carcinoma with medullary aspect	0 (0%)	2 (5.4 %)	1 (2.3 %)	
Mucinous carcinoma	0 (0%)	1 (2.7 %)	2 (4.5 %)	
Invasive micropapillary carcinoma	0 (0%)	2 (5.4 %)	0 (0%)	
Metaplastic carcinoma of non-specific type	1 (6.3 %)	1 (2.7 %)	0 (0%)	
Lobular carcinoma in situ	1 (6.3 %)	1 (2.7 %)	0 (0%)	
Invasive papillary carcinoma	0 (0%)	0 (0%)	2 (4.5%)	
Carcinoma with apocrine differentiation	1 (6.3 %)	0 (0%)	0 (0%)	
Tumor size				
T1 : ≤2 cm	3 (18.8 %)	8 (21.6 %)	10 (22.7 %)	>0.05
T2 : 2 cm <t<5 cm	9 (56.3 %)	16 (43.2 %)	19 (43.2 %)	
T3 : ≥5 cm	4 (25 %)	13 (35.1 %)	15 (34.1 %)	
Histo-pronostic grade				
SBR I	6 (37.5 %)	6 (16.2 %)	0 (0 %)	0.0001
SBR II	8 (50 %)	30 (81.1 %)	0 (0 %)	
SBR III	2 (12.5 %)	1 (2.7 %)	44 (100 %)	
Estrogen receptor status				
Positive	14 (87.5 %)	12 (32.4 %)	3 (8.8 %)	0.0001
Negative	2 (12.5 %)	25 (67.6 %)	31 (91.2 %)	
Status of progesterone receptors				
Positive	9 (56.3 %)	16 (43.2 %)	11 (32.4 %)	>0.05
Negative	7 (43.8 %)	21 (56.8 %)	23 (67.6 %)	
HER 2 status				
Positive	0 (0 %)	6 (17.1 %)	37 (86 %)	0.0001
Negative	13 (100 %)	29 (82.9 %)	6 (14 %)	
Status of lymph node metastases				
Positive	5 (45.5 %)	20 (66.7%)	25 (67.6 %)	>0.05
Negative	6 (54.5 %)	10 (33.3 %)	12 (32.4 %)	
Status of vascular emboli				
Presence	6 (37.5 %)	18 (51.4 %)	15 (34.1 %)	>0.05
Absence	10 (62.5 %)	17 (48.6 %)	29 (65.9 %)	

Loss of B antigen expression was observed in SBR grade 1 tumors with a percentage of 50% (6/12), SBR grade II with a percentage of 78.9% (30/38) and SBR grade III with a percentage of 95.75% (45/47). The cases expressing the H antigen were all (100%) grade SBR III (p = 0.0001).

Profile 3 corresponding to tumors not expressing B antigen and expressing H antigen, presented a percentage of 91.2% (31/34) of estrogen receptor negative. These receptors were absent in 78.9% (56/71) of the patients with loss of B antigen expression, presenting profiles 2 and 3 (p = 0.0001).

In order to verify by transitivity the association of estrogen receptor expression in the different stages of breast cancer with the expression of B and H antigens on the tumor tissue, a statistical analysis concerned the estrogen receptor status and the different histoprognostic grades SBR. Estrogen receptors are less expressed on SBR III tumors than on tumors of other grades. The percentage of positive estrogen receptor expression decreased with increasing histoprognostic SBR grade (p<0.0001).

Patients with loss of B-antigen expression showed HER2 overexpression in 86% (37/43) of tumors, and all B-antigen expressing tumors showed negative HER2 overexpression status (p=0.0001).

In order to verify by transitivity the association of HER2 receptor expression in the different SBR stages of breast cancer with the expression of B and H antigens, a statistical analysis concerned the HER2 receptor status and the different SBR histoprognostic grades. HER2 receptors are expressed by a higher number of SBR III tumors than tumors of other grades. The percentage of positive HER2 expression increased with increasing histoprognostic SBR grade (p=0.0001).

Node invasion was mainly observed in tumors not expressing the B antigen, with a percentage of 67.2% (45/67). However, this association is not statistically significant.

The associations between aberrant B antigen expression, age at diagnosis, histological types of carcinoma, tumor size, progesterone receptor status, and vascular emboli status showed no statistically significant associations.

In our sample, triple-negative tumors represent 22.22% (18/81) of all tumors with information regarding hormone receptor status and HER2 receptors. The expression profiles of B and H antigens on triple

negative and non-triple negative tumors are shown in Table 4.

Table 4: Expression profiles of B and H antigens on triple-negative and non-triple-negative tumors.

	Expression profiles of B and H antigens		
Type of carcinoma	**Profile 1:** B: Positive H: Negative	**Profile 2:** B: Negative H: Negative	**Profile 3:** B: Negative H: Positive
Triple negative	1 (1.23 %)	17 (20.99 %)	0 (0%)
Non-triple negative	12 (14.81 %)	18 (22.22 %)	33 (40.74 %)
$X^2 = 25.075$	**ddl = 2**		**p = 0.001**

B antigen was expressed in only one triple negative tumor. 94.44% (17/18) of triple negative tumors expressed neither B nor H antigen. The H antigen was not expressed on any triple negative tumor.

Table 4 shows that the distribution of B and H antigen expression on triple negative and non-triple negative tumors is statistically significant (p = 0.0001).

3. DISCUSSION

The immunohistochemical study revealed three patterns of B and H antigen expression on breast tumor tissue. Profile 1 corresponds to the expression of the B antigen and the non-expression of the H antigen. It represents 16.5% (16/97). Profile 2 corresponds to the non-expression of both B and H antigens and represents 38.1 % (37/97). Profile 3 corresponds to tumors not expressing the B antigen and expressing the H antigen and represents 45.4 % (44/97).

The association between the expression of B and H antigens and the histoprognostic grade showed that SBR grade I was more common in profile 1 tumors. SBR grade II has a positive association with profile 2. Profile 3 tumors are all SBR grade III. These observations confirm the results of Zouine et al, obtained by analyzing tumor tissues of the four blood groups of the ABO system.[9]

In addition to the obvious associations between each ABH antigen expression profile and histoprognostic grade, these findings suggest that the loss and aberrant expression of ABH antigens follows the progression of cancer and histoprognostic grades.[11]

In parallel to these findings, estrogen receptor expression is initially high in tumors expressing the B antigen and then low in tumors expressing the H antigen alone. The same observation is made concerning the expression of estrogen receptors which progressively decreases with the histoprognostic grade (from SBR I to SBR III).

Similarly, the percentage of HER2-expressing tumors changes progressively with SBR grade. Indeed, it is nil (0/9) in SBR I tumors, 14% (5/36) in SBR II tumors, and 83% (38/46) in SBR III tumors. This gradient of HER2 evolution is also observed in the association with B and H antigen expression: profile 1 is superimposed on SBR grade I, profile 2 on SBR grade II, and profile 3 on SBR grade III.

This evolution of B and H antigen expression indicates that they are expressed in a normal way (B-Ag: positive; H-Ag: negative) on low-grade tumors with a high expression of estrogen receptors and a low expression of HER2 receptors. Taking into account the different associations, it appears that the decrease of ABH antigen expression in tumor cells is proportional to the high expression of HER2, and the low expression of estrogen receptors. Therefore, the loss of B and H antigen expression and subsequent re-expression of H follows the progression of the cancer to a poor prognosis (Table 5).[12]-[14]

Table 5: Expression profiles of B and H antigens according to major prognostic factors.

Expression profiles of Ag B and H	Majority SBR grade	Percentage of tumors with positive ER	Percentage of tumors with HER2 positive	Prognosis
Profile 1 (B+ ; H-)	SBR I (Low)	87.5 % (High)	0 % (Low)	Good
Profile 2 (B- ; H-)	SBR II (Medium)	32.4 % (Medium)	17.1 % (Medium)	Medium
Profile 3 (B- ; H+)	SBR III (High)	8.8 % (Low)	86 % (High)	Bad

These progressive aberrations suggest several probabilities regarding the evolution of tumor expression of ABH antigens. The process would start at a phase between the 1st and 2nd grade SBR, by the loss of the successive expression of the B antigen then H, or both simultaneously, then the re-expression or the neo-expression of the H antigen. Indeed, during the tumor process many genetic abnormalities can occur. The absence of ABH antigens could be due, among other things, to the repression or deletion of the FUT1 gene, caused by an as yet unknown genetic event, such as a partial or total deletion of the gene or a CpG hyper-methylation of the regulatory sequence of the gene. In the case of FUT1 gene repression, a possible reactivation could occur that would explain the reexpression of the H antigen on SBR III grade tumors observed in our work.

The relevance of this reactivation assumption is countered by the absence of B antigen reexpression from its H precursor.[15], [16]

This limitation would favor the likely hypothesis of partial or total deletion of the FUT1 gene encoding the H antigen. The deletion could also affect both FUT1 and ABO genes simultaneously.

In the case of gene deletion, the appearance of the H antigen on SBR III grade tumors is only possible by a neo-expression due to another gene of the fucosyltransferase (FUT) family, thus probably another H antigenic form expressed specifically on SBR III stage tumor cells, and not allowing the catalysis of the B antigen by α(1,3)galactosyltransferases.

Indeed, there are several FUT genes: FUT1, FUT2, FUT3, FUT5 and FUT6 located on chromosome 19, FUT4 on chromosome 11, FUT7 on chromosome 9, FUT8 on chromosome 14, FUT9 on chromosome 6, FUT10 on chromosome 8, FUT11 on chromosome 10, FUT12 and FUT13 are located on chromosome 20 and 21 respectively.[17]-[21]

Furthermore, alteration of one gene can cause changes in another, through epigenetic modifications, such as the expression of CD44 isoforms containing exon V6 (CD44 V6) and CD-133, which have been observed in co-expression with the H antigen at metastatic stages of tumors. These markers are cancer-initiating molecules and indicate an unfavorable evolution. Indeed, CD44 activates transcription factors and genes NANOG, SOX2 and OCT4 which are very active in stem cells, i.e. in immature cells. This is also corroborated by the overexpression of the growth factor receptor HER2, involved in carcinogenesis and invasiveness.[16], [22]-[27]

The lymph node involvement and its association with the H-antigen profile is not statistically significant in our series, despite their associations with a dominant percentage of 67.2% of cases. According to Léon et al, the loss of ABH antigens facilitates metastatic dissemination by increasing cell motility and resistance to apoptosis. In-vitro studies have evoked α (1-2) fucosylation as a tumorigenicity-enhancing feature in rat colon cancer cells. This feature is thought to be based on the ability of H antigen-expressing malignant cells to escape immune control by influencing their recognition by Natural Killer cells and lymphokine-activated cytotoxic cells.[28]-[30]

Our hypothesis of the evolution of ABH antigen expression with the tumor process and clinical worsening can only be proven by prospective studies on breast cancer patients, which is ethically not feasible, or by in-vitro studies by culture of cancer cells whose biological process of transformation is difficult to control.

Breast tumors with negative estrogen receptor, progesterone receptor, and HER2 receptor status, commonly called triple-negative tumors, represent 22.22% of our sample. Immunohistochemical study of these carcinomas showed loss of B antigen expression with non-expression of H antigen in 94.44% of tumors. This subtype of breast cancer is associated with an unfavorable clinical profile with a high risk of early metastatic relapse due to the aggressive nature of these tumors, their partial response to chemotherapy and the lack of targeted therapies used in clinical practice. In the absence of research exploring the expression of ABH antigens in this type of breast carcinoma, the present study is limited by the small number of cases (18 patients).[31]-[37]

Before concluding, as for the results and discussion of our work, it is important to point out that despite all the statistical evidence regarding the involvement of the ABO system in human physiology and pathophysiology, the mechanisms by which it may interact with the development and progression of cancer, are still poorly understood. However, further studies are needed to identify the exact mechanisms by which ABO blood types may influence the incidence and progression of breast cancer, which will lead to consistent and concrete results.

4. CONCLUSION

In conclusion, like many similar studies, this research also has its own limitations, including its monocentric and retrospective nature, and the number of cases limited by the absence of clinical record information, regarding ABO blood type, and the status of different prognostic factors. Nevertheless, taking into account all our results described and analyzed, it appears that ABH aberrant expression is an early event that could have a prognostic value, as abnormalities in the expression of ABO system antigens are due to altered glycosylations characteristic of tumor cells, and may appear early in the healthy tissue surrounding the tumor.

Aberrant expressions, including loss of ABH antigens, are related to the initial oncogenic transformation, and may precede the specific symptomatology of malignant conversion.

CONFLICT OF INTEREST

The authors declare that they haven't known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

FUNDING SOURCES

The research has not been externally funded.

REFERENCES

1. S. C. Rocha-Brischiliari et al., « The Rise in Mortality from Breast Cancer in Young Women: Trend Analysis in Brazil », PLOS ONE, janv. 2017; 12(1): e0168950.
2. B. A. Almutlaq et al., « Breast cancer in Saudi Arabia and its possible risk factors », J. Cancer Policy, juin 2017; 12: 83‑89.
3. E. Almagro, C. S. González, et E. Espinosa, « Prognostic factors of early breast cancer », Med. Clínica, févr. 2016; 146(4): 167‑171.
4. S. K. Rummel et R. E. Ellsworth, « The role of the histoblood ABO group in cancer », Future Sci. OA, mars 2016; 2(2):
5. X. Cao, Z.-S. Wen, Y.-J. Sun, Y. Li, L. Zhang, et Y.-J. Han, « Prognostic value of ABO blood group in patients with surgically resected colon cancer », Br. J. Cancer, juill. 2014; 111(1): 174‑180.
6. R. Mitra, N. Mishra, et G. P. Rath, « Blood groups systems », Indian J. Anaesth, 2014; 58(5): 524‑528.
7. A. K. Panda, S. K. Panda, A. N. Sahu, R. Tripathy, B. Ravindran, et B. K. Das, « Association of ABO blood group with severe falciparum malaria in adults: case control study and meta-analysis », Malar. J, oct 2011; 10(309).
8. S. Zouine et al., « Immunohistochemical and genetic exploration of incompatible A blood group antigen expression in invasive micropapillary breast carcinoma: A case report », /data/revues/24523186/v65i2/S2452318617300156/.
9. S. Zouine et al., « Expression of Histo-blood Group Antigens in Tumor and Adjacent Normal Breast Tissues as Prognostic Markers of Breast Carcinoma », J. Breast Cancer, févr. 2020; 23(1): 69‑79.
10. O. F. Rasmussen et L. Rudbeck, « Immunohistochemistry: A Dako Perspective », in Handbook of Practical Immunohistochemistry, Springer, New York, NY, 2015; 57‑67.
11. G. Arpino, M. Milano, et S. De Placido, « Features of aggressive breast cancer », Breast Edinb. Scotl, oct. 2015; . 24(5): 594‑600.
12. S. Saxena et K. K. Gupta, « Association of ABO Blood Groups in Relation to Gynaecological Cancers in Western Rajasthan. - », Int. J. Health Sci. Res. IJHSR, 2016; 6(5): 118‑124, Consulté le: févr. 18, 2018.
13. S. Saxena, V. K. Chawla, K. K. Gupta, et K. L. Gaur, « Association of ABO blood group and breast cancer in Jodhpur », Indian J. Physiol. Pharmacol, mars 2015; 59(1): 63‑68.
14. S. Rummel, C. D. Shriver, et R. E. Ellsworth, « Relationships between the ABO blood group SNP rs505922 and breast cancer phenotypes: a genotype-phenotype correlation study », BMC Med. Genet, mai 2012; 13(41).
15. Y. Koda, M. Soejima, et H. Kimura, « The polymorphisms of fucosyltransferases », Leg. Med. Tokyo Jpn, mars 2001; 3(1): 2‑14.
16. E. A. McSherry, S. Donatello, A. M. Hopkins, et S. McDonnell, « Molecular basis of invasion in breast cancer », Cell. Mol. Life Sci. CMLS, déc. 2007; 64(24): 3201‑3218.
17. G. Fabini, A. Freilinger, F. Altmann, et I. B. H. Wilson, « Identification of Core α1,3-Fucosylated Glycans and Cloning of the Requisite Fucosyltransferase cDNA from Drosophila melanogaster POTENTIAL BASIS OF THE NEURAL ANTI-HORSERADISH PEROXIDASE EPITOPE », J. Biol. Chem, juill. 2001; 276(30): 28058‑28067.
18. D. Rendić, A. Linder, K. Paschinger, N. Borth, I. B. H. Wilson, et G. Fabini, « Modulation of Neural Carbohydrate Epitope Expression in Drosophila melanogaster Cells », J. Biol. Chem, oct. 2006; 281(6): 3343‑3353.
19. M. Yamamoto-Hino et al., « Phenotype-based clustering of glycosylation-related genes by RNAi-mediated gene silencing », Genes Cells, juin 2015; 20(6): 521‑542.
20. C. Roos, M. Kolmer, P. Mattila, et R. Renkonen, « Composition of Drosophila melanogaster Proteome Involved in Fucosylated Glycan Metabolism », J. Biol. Chem, janv. 2002; 277(5): 3168‑3175.
21. V. Lombard, H. Golaconda Ramulu, E. Drula, P. M. Coutinho, et B. Henrissat, « The carbohydrate-active enzymes database (CAZy) in 2013 », Nucleic Acids Res., vol. 42, no Database issue, janv. 2014; D490-495.
22. R. Mejías-Luque, A. López-Ferrer, M. Garrido, À. Fabra, et C. De Bolós, « Changes in the invasive and metastatic capacities of HT-29/M3 cells induced by the expression of fucosyltransferase 1 », Cancer Sci, juill. 2007; 98(7): 1000‑1005.
23. J.-M. Zhang, K. Wei, et M. Jiang, « OCT4 but not SOX2 expression correlates with worse prognosis in surgical patients with triple-negative breast cancer », Breast Cancer Tokyo Jpn, mars 2018.
24. R. Zhang, J. Jiao, H. Chu, H. Yang, L. Wang, et N. Gao, « Expression of microRNA-145, OCT4, and SOX2 in double primary endometrioid endometrial and ovarian carcinomas », Histol. Histopathol, mars 2018; 11986.
25. S. Gong, Q. Li, C. R. Jeter, Q. Fan, D. G. Tang, et B. Liu, « Regulation of NANOG in cancer cells », Mol. Carcinog, sept. 2015; 54(9): 679‑687.
26. W.-M. Lin, U. Karsten, S. Goletz, et Y. Cao, « Carbohydrate Antigens as Cancer-Initiating Cell Markers », 2011.
27. M. Kryworuckho, F. Diaz-Mitoma, et A. Kumar, « CD44 isoforms containing exons V6 and V7 are differentially expressed on mitogenically stimulated normal and Epstein-Barr virus-transformed human B cells. », Immunology, sept. 1995; 86(1): 41‑48.
28. P. León-Atance et al., « Prognostic influence of loss of blood group A antigen expression in pathologic

stage I non-small-cell lung cancer », Arch. Bronconeumol, févr. 2012; 48(2): 49‑54.
29. S. Hakomori, « Structure, organization, and function of glycosphingolipids in membrane », Curr. Opin. Hematol, janv. 2003; 10(1): 16‑24.
30. M. Xu, F. Wang, J. C. Gildersleeve, et S. Achilefu, « MAb L9E10 to blood group H2 antigen binds to colon cancer stem cells and inhibits tumor cell migration and invasion », Hybrid. août 2010, 2005; 29(4): 355‑359.
31. S. Park et al., « Prognostic value of ABO blood types in young patients with breast cancer; a nationwide study in Korean Breast Cancer Society », Med. Oncol. Northwood Lond. Engl, juin 2017; 34(6): 118.
32. M. Franchini, G. M. Liumbruno, et G. Lippi, « The prognostic value of ABO blood group in cancer patients », Blood Transfus, sept. 2016; 14(5): 434‑440.
33. S. Zouine et al., « ABO blood groups in relation to breast carcinoma incidence and associated prognostic factors in Moroccan women », Med. Oncol. Northwood Lond. Engl, juill. 2016; 33(7): 67.
34. J. Yu, F. Gao, V. S. Klimberg, et J. A. Margenthaler, « ABO blood type/Rh factor and the incidence and outcomes for patients with triple-negative breast cancer », Ann. Surg. Oncol, oct. 2012; 19(10): 3159‑3164.
35. E. A. Rakha, M. E. El-Sayed, A. R. Green, A. H. S. Lee, J. F. Robertson, et I. O. Ellis, « Prognostic markers in triple-negative breast cancer », Cancer, janv. 2007; 109(1): 25‑32.
36. C. Liedtke et al., « Response to neoadjuvant therapy and long-term survival in patients with triple-negative breast cancer », J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol, mars 2008; 26(8): 1275‑1281.
37. A. A. Onitilo, J. M. Engel, R. T. Greenlee, et B. N. Mukesh, « Breast cancer subtypes based on ER/PR and Her2 expression: comparison of clinicopathologic features and survival », Clin. Med. Res, juin 2009; 7: 1‑2, 4‑13.