The Association and Relation of ABO Blood Group and Secretor status with the Breast Cancer.

1Lyden K. Mohammed, 2Khalid Mahdi Salih, 3RanaS.Jawad, 4Salim R. Al – Aubaidy, 5Mohammed Yousef

1Baghdad University, Al-Khawarzmy Engineering College, Department of Biomedical
2Al-Mustansiriya University, College of Science, Department of Biology
3Baghdad University, College of Medicine, Department of Pathology
4Assuit University

ABSTRACT
The burden of breast cancer in both developed and developing countries, and in many regions of the world, it is the most frequently occurring malignant disease in women, comprising 18% of all female cancers, and worldwide, breast cancer is the fifth most common cause of cancer mortality. The present study was planned with the aims to shed light on association between ABO(H) and Secretor status with breast cancer. The study involved 181 Iraqi women, who were distributed into two groups of patients and one group of healthy control. The patients were referred to the hospitals for early detection of breast cancer and gynecology from different site during the period from July 2013 to October 2014. The results showed that about one half of malignant breast (MB) women were from group A (51.4%) in comparison with 42% of normal breast (NB) women and 35% of benign breast (BB) women, the majority of women in NB and BB groups were secretors (72% and 60% respectively), while the majority of MB women (61.3%) were non-secretors with high significant difference (P < 0.0003). It can be concluded that the frequency of group A constitutes high frequency among MB women when compared as alone with other groups, about two third of malignant breast women were non-secretors (61.3%), while only 28% and 40% of women with normal and benign breast diseased respectively were non-secretors.

KEYWORDS: Breast cancer, Blood group, Secretor status.

INTRODUCTION
The burden of breast cancer in both developed and developing countries, and in many regions of the world, it is the most frequently occurring malignant disease in women, comprising 18% of all female cancers, and worldwide, breast cancer is the fifth most common cause of cancer mortality [1].

According to the Iraqi Cancer Registry data during the period 2000-2009, a total of 23,792 incident breast cancer cases were registered among females aged ≥15 years, represented 33.8% of all cancers. The incidence rate of all female breast cancer in Iraq (all ages) increased from 26.6 per 100,000 in 2000 to 31.5 per 100,000 in 2009, which became one of the major threats to Iraqi female health [2].

The first suggestion of an association between ABO blood group antigens and malignancy was made almost 100 years ago, yet the role of the ABO blood group in cancer risk and prognosis remains controversial[3]. ABO blood groups are a stable feature of a population and they differ among various socioeconomic, geographical and ethnic groups. In Europe, highest frequency is of allele A, increasing to allele B from West to East [4]. Many risk factors are associated with the development of breast cancer, it is seldom mentioned that blood type has an influence on susceptibility and outcomes. In fact, some researchers have even gone so far as to say that
"blood groups were shown to possess a predictive value independent of other known prognostic factors" when discussing breast cancer. Other researchers have actually suggested that a degree of the susceptibility to breast cancer, from a gene perspective, might be a result of a breast cancer-susceptibility locus linked to the ABO locus located on band q34 of chromosome 9 [5].

Individuals could be classified as ‘secretors’ and ‘nonsecretors’ according to their ability to secrete ABO blood group antigens in saliva [6]. ABO blood group antigens (A, B, and H), in addition to their presence on blood cells and platelets, are also present on other tissue cells and are variably expressed through body fluids, such as saliva, tears, semen, urine, gastric juice, and breast milk, depending on whether the individual possesses the secretor gene or not, the inherited A, B, O genes, and Lewis blood group system [7].

The secretor gene encodes for enzymes (glycosyltransferases), which become active in mucin-secreting cells like goblet and mucous cells of mucous membranes and different glands, resulting in the secretion of the corresponding blood group antigens in the body fluids [8]. H antigen that is present on the cells of individuals with O blood group is the base for A and B antigens, but A and B antigens differ only in their added terminal sugars, which are controlled by specific enzymes called transferase enzymes. These enzymes are under the control of inherited genes, which are A, B, H (FUT1) genes and secretor (FUT2) genes [9].

**MATERIALS AND METHODS**

Subjects and Samples:
This study has been designed upon 181 Iraqi women in different sites and hospitals for early detection of breast cancer and gynecology during the period from July 2013 to October 2014. Women involved in this study include 50 women with normal breast (NB), and 131 breast diseased [111 from them are with malignant breast cancer (MB) and 20 with benign-breast disease (BB)], the diagnosis was confirmed according to the fine needle aspiration (FNA) technique carried out by specialists.

Two milliliters of peripheral blood were aspirated by using vein punctures and dispensed in an EDTA tube. Saliva was collected from all subjects (after rinsing their mouth 2 times with cold drinking water 5-10 minutes prior to collection); about 3-5 ml of saliva was transferred into sterile container. All samples centrifuged at 3000 rpm for 10 minutes to eliminate any debris.

Determination of ABO:
According to tube method [10] and by using 3-5% RBCs suspension, anti-A and anti-B kit from (Biotec, Germany), ABO blood groups were determined. Similarly, Lewis blood groups were determined according to tube method [11].

Determination of Secretory status:
Anti-H lectin (Biorex, UK) is used to resolve problems of ABO subgroups by determining the degree of H reactivity on the red blood cells in agglutination tests and for determining the ABH secretor status, particularly in group (O) individuals, by inhibition tests using saliva. Tube technique [15] was carried out by collecting 2-3 ml of saliva which placed in a bath of boiling water for 10-15 minutes and centrifuged at 3000 rpm for 2 minutes and separated the supernatant by pasture pipette which is directly used for this test. The test was provided by adding of one drop of appropriately diluted blood grouping reagent (Anti-A, Anti-B, or Anti-H) and one drop of patient’s saliva. After incubation for 10 minutes at room temperature, 2 drops of 3-5% suspension of washed indicator red cells were added. The tube incubated for 30 minutes and centrifuged again, then gently shaken the tube and macroscopically detecting the agglutination as matt. Agglutination of indicator cells by antibody in tubes containing saliva indicates that the saliva does not contain the corresponding antigen (non-secretor). Whereas, failure of known antibody to agglutinate indicator cells after incubation with saliva indicates that the saliva contains the corresponding antigen and considered as secretor.

Statistical analysis:
Descriptive data were expressed as percentage values, whereas differences among groups were analyzed by using Pearson Chi-square test. The P value of differences < 0.05 were considered significant.

Results:
The distribution of blood groups according to ABO system showed no significant differences in the occurrence of blood groups A, B, AB, and O among women of the three major groups (Table 1). However, about one half of MB women were from group A (51.4%) in comparison with 42% of NB women and 35% of BB women.
Table 1: Distribution of blood groups according to ABO & Lewis systems

<table>
<thead>
<tr>
<th>Groups</th>
<th>ABo system</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>B</td>
<td>AB</td>
</tr>
<tr>
<td>Normal Breast (N= 50)</td>
<td></td>
<td>21</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(42%)</td>
<td>(6%)</td>
<td>(2%)</td>
</tr>
<tr>
<td>Benign Breast (N= 20)</td>
<td></td>
<td>7</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(35%)</td>
<td>(5%)</td>
<td>(5%)</td>
</tr>
<tr>
<td>Malignant Breast (N=111)</td>
<td></td>
<td>57</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(51.4%)</td>
<td>(9%)</td>
<td>(3.6%)</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chi-square (P value)</td>
<td></td>
<td>$X^2 = 5.9$</td>
<td>($P &gt; 0.05$)</td>
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</tr>
</tbody>
</table>

The secretory status of all women was initially determined by hemagglutination inhibition test Table 2 revealed that the majority of women in NB and BB groups were secretors (72% and 60% respectively), while the majority of MB women (61.3%) were non-secretors with high significant difference ($P < 0.0003$).

Table 2: Frequency of secretor & non-secretor women according to inhibition of hemagglutination tests

<table>
<thead>
<tr>
<th>Groups</th>
<th>Secretor</th>
<th>Non-secretor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Breast (N= 50)</td>
<td>36 (72%)</td>
<td>14 (28%)</td>
</tr>
<tr>
<td>Benign Breast (N= 20)</td>
<td>12 (60%)</td>
<td>8 (40%)</td>
</tr>
<tr>
<td>Malignant Breast (N=111)</td>
<td>43 (38.7%)</td>
<td>68 (61.3%)</td>
</tr>
<tr>
<td>Chi-square= 16.11 (P value)</td>
<td>$&lt; 0.0003$</td>
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Discussion:

Although this study didn’t find significant differences in the distribution of ABO blood groups among NB, BB, and MB women (Table 1), the frequency of group A constitutes high frequency among MB women when compared as alone with other groups. In addition to these results Table 2found that about two third of malignant breast women were non-secretors (61.3%), while only 28% and 40% of women with normal and benign breast diseases respectively were non secretors. Therefore, the secretory status as a genetic trait constitute a strong non-preventable risk factor for breast cancer.

Although ABO blood group has been linked with risk for several tumor types, the biologic mechanisms underlying these associations remain uncertain. The ABO gene encodes a glycosyltransferase with three main variant alleles (A, B, and O) with different substrate specificities [Reid and Mohandas, 2004]. The A, B, and O glycosyltransferases transfers N-acetylgalactosamine, D-galactose, or no sugar residue, respectively, to a protein backbone known as the H antigen [Yazer, 2005]. H antigen that is present on the cells of individuals with O blood group is the base for A and B antigens, which are controlled by specific enzymes called transferase enzymes by adding different terminal sugars to A and B antigens. These enzymes are under the control of inherited genes, which are A, B, H (FUT1) genes and secretor (FUT2) genes [9]. H (FUT1) and secretor (FUT2) genes are separate but closely linked.

Blood group antigens are expressed on the surface of red blood cells and numerous other tissues throughout the body, including breast ductal and lobular cells [14,15]. Alterations in ABO antigen expression on the surface of malignant cells, compared to normal epithelium, have been seen for a variety of tumor types, including breast cancer [16]. Modified expression of blood group antigens on the surface of cancer cells may alter cell motility, sensitivity to apoptosis, and immune escape, with important implications for malignant progression [17].

The secretor phenotype is defined by the FUT2 gene, a fucosyltransferase that catalyzes the addition of terminal fucose residues to produce the H antigen, an acceptor to which the ABO transferase adds its glycosyl. A functioning FUT2 enzyme allows for the secretion of ABO antigens into body fluids, however, homozygous inactivating mutations in FUT2 occur in approximately 20% of non-secretor individuals [18]. It has been found that ABH and Lewis antigen expression has been associated with cancer development and prognosis, tumor differentiation, and metastasis. Non-secretor genotype was associated with axillary lymph node metastasis and could be useful to predict respectively breast cancer susceptibility and axillary lymph nodes metastasis.

REFERENCES