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Determining the Expression Rate of the IgM and IgA in B Cell Lymphomas by Immunohistochemistry

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Abstract: This study aimed at assessing the expression rate of IgA and IgM in B cell lymphoma immunohistochemically. Paraffin-embedded specimens of NHL evaluated for presence of CD20 by immunohistochemical staining and then, the positive cases were again immunohistochemically assessed for expression of IgM and IgA. Fifty two paraffin-embedded specimens evaluated. Thirty six cases (69.2%) were positive for the CD20. The mean age of these patients (CD20+ cases) was 50.0±23.6 years. Twenty two cases (61%) were male and 14 cases (39%) were female. The frequency of the IgM+, IgA+ and IgA/IgM+ (simultaneous) cases was 15(41.7%), 9(25%) and 4(11.1%), respectively. The current rates are in the range of similar reports in the literature.

Key words: B-cell lymphoma, immunohistochemistry staining, IgM, IgA

INTRODUCTION

Non-Hodgkin Lymphoma (NHL) is one of the most common malignancies occurring worldwide. This malignancy is a kind of lymphoid cell neoplasm presenting as a tissue mass. It is classified according to the lymphocyte cell type (B or T) and also the cell surface markers. Surface immunoglobulins (Igs) are the diagnostic markers of the B cell lymphocytes and considered as the surface antigens. Their expression is variable. In the late two decades, has occurred a significant progress in the field of immunology and molecular biology which has increased our knowledge of the origin and function of the lymphocytes. This has been even led to structural changes in nomination and categorizing methods for the lymphomas (Ioachim and Ratech, 2002). Using innovative and new techniques, it is now much more precise and rapid to recognize the T and B lymphocytes and determine their subgroups by detection of the superficial markers and the secretory products (Cotran *et al*., 1999). This achievement seems more outstanding when we know that the discrimination of these cells may be quite cumbersome and time-consuming by the microscopic and morphological-based methods. The B lymphocytes differentiate in the bone marrow and other lymphatic organs like lymph nodes. The superficial immunoglobulins are the receptors of the B cells which have been used as a marker of recognition (Ioachim and Ratech, 2002). Immunophenotyping is a technique which can help in differentiating lymphomas from the similar reactive conditions and even discrimination between the B cell and T cell lymphomas (Mills *et al*., 2004). It has been previously reported that the B cell lymphomas express the immunoglobulin M (IgM) in majority of cases and sometimes, the immunoglobulin A (IgA). Presence of the both immunoglobulins (IgM/IgA) at the same time is estimated to be in less than 5% of the cases (Sitia *et al*., 1981). The frequency of IgA+, IgM+ and IgA/IgM+ cases was reported as high as 75, 25 and 25%, respectively in another series (Bende *et al*., 2003). The frequency of IgA+, IgM+ and IgA/IgM+ cases was 53.3, 13.3 and 13.3%, respectively in another study by Inagaki *et al*. (2002). Tada and Hämmerling (1980) reported a rate of 5-6% for the simultaneous presence of IgA and IgM in the B-cell lymphoma. In a study by Aarts *et al*. (2002), the expression rate of the IgM was 100%. Strauchen and Mandeli (1991) reported a rate of 74% for the IgM+ specimens. It was 70.6% in a series by Andriko *et al*. (2001) and 75% in another study by Dominis *et al*. (2002). This study aimed at determining the rate of IgM and IgA expression in the B-cell lymphomas by an immunohistochemical staining method. The results of the current study may be of great importance because there is limited number of studies in this regard of which some reports are very old. Likewise, the available data is almost drawn from the Western populations which may be different from that in the target population in the current study.

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MATERIALS AND METHODS

In an analytic-descriptive study, biopsy specimens of Non-Hodgkin's Lymphoma (NHL) were evaluated for presence of CD20 (Pan-B cell marker). Fifty two specimens were assessed. Thirty six cases were positive for CD20 out of the 52 primary specimens. Further evaluation was performed on theses 36 cases for determining the presence (expression) of IgA and/or IgM by the immunohistochemical staining technique. This study performed during a one-year period (2008-2009) in the Pathology Department of Imam Hospital, Tabriz, Iran. Formalin fixed paraffin embedded blocks of specimens with definite histopathological diagnosis of NHL were used. Samples from the follicular and paracortical zones in the normal lymph nodes were used as the positive and negative controls, respectively. The specimens were dissected in 4 micron slices. The immunohistochemical staining was performed in two level: first for the presence of C20 (as mentioned before); and second for presence of IgM and/or IgA on the CD20+ specimens from the first level. The method of staining was Avidin-biotin-peroxidase complex immunohistochemistry in these stages: deparaffinization (exposure the specimen to 60°C for 1 h), rehydration (using distilled water, 100% alcohol, Xylene), inactivation of the endogenous peroxidase (by 3% H2H2 plus methanol), washing with water, transferring the specimens to the Phosphate Buffered Saline (PBS) with the pH of 7.2, incubation with specific antibodies against the CD20 and then, antibodies against the IgM and IgA in the room temperature for one hour, washing with the PBS, adding specific secondary antibodies, washing with the PBS, adding 3,3-diamino Benzidine substrate and finally, staining with the Gills-hematoxyline. Finally, the specimens were evaluated under the light microscope. Cytoplasmic membrane staining >10% was considered as a positive result. This study was approved by the University Ethics Committee. The data has shown as Mean±SD and frequency (percent). The SPSS software version 15 was used for statistical comparisons. The Contingency tables (Chi-square or Fisher's Exact tests, when appropriate) were used for analyzing the data. The p value ≤0.05 was considered statistically significant.

RESULTS

Fifty two specimens of NHL were evaluated. Thirty six cases (69.2%) were positive for CD20. The mean age of these patients was 50.0±23.6 (5-80) years. Twenty two cases (61%) were males and 14 cases (39%) were females. Two cases (5.5%) were under 10 years, 4 cases (11.1%) between 10 and 19 years, 4 cases (11.1%) between 20 and 29 years, 1 case (2.8%) between 30 and 39 years, 5 cases (13.9%) between 40 and 49 years, 4 cases (11.1%) between 50 and 59 years, 5 cases (13.9%) between 60 and 69 years and 11 cases (30.6%) 70 years or older. Sixteen cases (44.4%) were from neck, 9 cases (25%) from mediastinum, 3 cases (8.3%) from axillary region, 3 cases (8.3%) from intestine and 1 case (2.8%) from stomach, thyroid gland, liver, nose and tongue, each one. IgM and IgA were positive in 15 and 9 cases, respectively (Fig. 1). IgM and IgA were simultaneously positive in 4 cases (Fig. 1). IgM was positive in 6 cases (27.2%) in the male group and in 9 cases (64.3%) in the female patients. The frequency of IgM+ cases was significantly higher in the females ($p = 0.028$). IgA was positive in 4 cases (18.2%) in the male group and in 5 cases (35.8%) in the female group with no significant difference ($p = 0.267$). IgM and IgA was simultaneously positive in 1 case (4.5%) in the male group and in 3 cases (21.4%) in the female patients with no significant difference ($p = 0.277$). The results in different age groups are summarized in Table 1. According to Table 1, the frequency of IgM+ cases was significantly higher in the patients aged 70 years or older ($p = 0.025$) with no significant difference regarding the frequency of IgA+ ($p = 0.223$) and IgM/IgA+ cases ($p = 0.998$). The results according to the site of biopsy are summarized in Table 2. As seen, the frequency of IgM+ cases is higher in the specimens from neck, mediastinum, intestine, axillary region, thyroid gland and nose in a decreasing order. The frequency of IgA+ cases is higher in the specimens from neck, intestine, mediastinum and stomach in a decreasing order. IgM/IgA+ cases were present in the specimens from intestine and neck.

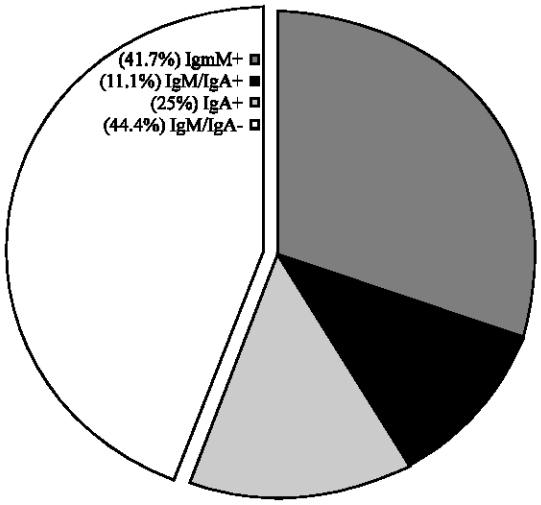

Fig. 1: Percentage of IgM+, IgA+ and IgM/IgA+ cells in the specimens with the B cell lymphoma

Table 1: Frequency of IgM, IgA and IgM/IgA positive cases in different age groups

Age group (year)	IgM+	IgA+	IgM/IgA+
≤10	1 (6.7)	1 (11.1)	1 (25)
10-19	0 (0)	3 (33.3)	0 (0)
20-29	2 (13.3)	0 (0)	2 (50)
30-39	0 (0)	0 (0)	0 (0)
40-49	0 (0)	4 (44.4)	0 (0)
50-59	0 (0)	0 (0)	0 (0)
60-69	4 (26.7)	0 (0)	0 (0)
70≤	8 (53.3)	1 (11.1)	1 (25)

Values in brackets indicate percentage

Table 2: Frequency of IgM, IgA and IgM/IgA positive cases in different sites of biopsy

Site	IgM+	IgA+	IgM/IgA+
Neck	4 (26.7)	4 (44.4)	1 (25)
Mediastinum	4 (26.7)	1 (11.1)	0 (0)
Stomach	0 (0)	1 (11.1)	0 (0)
Intestine	3 (20)	3 (33.3)	3 (75)
Axillary region	2 (13.)	0 (0)	0 (0)
Thyroid gland	1 (6.7)	0 (0)	0 (0)
Nose	1 (6.7)	0 (0)	0 (0)

Values in brackets indicate percentage

DISCUSSION

There is limited number of studies evaluating the superficial immunoglobulins in the B-cell lymphomas, quantitatively. This limitation is even more prominent in the A and M immunoglobulins and the immunohistochemical staining method in this regard. Sitia *et al.* (1981) evaluated two isotypes of IgA and IgM in the B-cell lymphoma. The rat B-cell lymphoma cells were extracted and cultured. The superficial immunoglobulins were assessed by the immunofluorescence staining method. Before culture, on day 7 and on day 15, the frequency of IgM+ cases were 70, 10 and 1.5%, respectively. The according rates were 25, 85 and 85% for the IgA+ cases, respectively. The frequency of IgM/IgA+ cases was 6, 6 and 1.5%, respectively. Bende *et al.* (2003) performed a similar study. They evaluated the cases with follicular lymphoma from the gastrointestinal tract origin and 4 cases were enrolled. The frequency of IgA+, IgM+ and IgA/IgM+ cases was 75, 25 and 25%, respectively. Inagaki *et al.* (2002) evaluated the specimens of marginal zone extra-nodal B-cell lymphoma with primary origin of thymus. Fifteen cases were assessed by immunohistochemical staining method, applying DAKO polyclonal antibody. The frequency of IgA+, IgM+ and IgA/IgM+ cases was 53.3, 13.3 and 13.3%, respectively. Tada and Hämmerling (1980) reported a rate of 5-6% for the simultaneous presence of IgA and IgM in the B-cell lymphoma. In another series by Aarts *et al.* (2002), the expression rate of the IgM was evaluated by the immunohistochemical staining method. This rate was 100%. Strauchen and Mandeli (1991) evaluated 345 specimens of the B-cell lymphoma by immunohistochemical staining method. IgM was present in 74% of the cases. The histopathological subtypes had different rate of expression, ranging from 59 to 100%. Andriko *et al.* (2001) evaluated 20 cases with lymphoblastic B-cell lymphoma. All the specimens were positive for CD20. The frequency of IgM+ cases was 70.6%. Dominis *et al.* (2002) reported a rate of 75% for the presence of IgM in the specimens of the diffuse large B-cell lymphoma. The frequency of IgA+ cases was reported to be higher. Summarizing the results of the different mentioned studies, the mean frequency of IgM+ cases was 48% (1.5-100%), the mean frequency of IgA+ was 49.6% (13.3-85%) and the mean frequency of IgA/IgM+ was 8.5% (1.5-25%). The corresponding rates in the current study were 41.7, 25% and 11.1%, respectively. Many causes of this inhomogeneity might be proposed. Type of the lymphoma may alter the expression rate of these immunoglobulins (Bende *et al.*, 2003; Strauchen and Mandeli, 1991). The stage of the cancer may also have a role in this regard. It has been shown that the expression rate of these immunoglobulins is higher in the low and intermediate stages comparing with the high stages of the lymphoma (Jaffe, 1999). The method different studies have been used in determining the expression of IgA/IgM is another important cause of discrepancy. The type of antibodies is also important in this regard (Inagaki *et al.*, 2002). And finally, it has been declared that the staining rate is higher in the fresh-frozen specimens comparing with the old archived ones (Rosai, 2004). Despite these differences between studies, the current results are rather similar to the others. As, there is no similar report in comparing the expression rate of IgA/IgM in the B-cell lymphomas relating to the gender and age of the patients, further studies are recommended in this regard.

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