



Original Article

Short and Long Term Effects of Splenectomy on Memory B Cell Level in Children

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Abstract

Introduction: Splenectomy has long been used to treat benign hematological abnormalities such as immune thrombocytopenia (ITP), some hemolytic anemias- especially hereditary spherocytosis and thalassemia, and prehepatic portal hypertension. The discovery that splenectomized individuals are more vulnerable to encapsulated organism infection has been attributed to the spleen's lack of filtration and the development of anticarbohydrate antibodies. Recent research in such splenectomized patients suggests that the lack of this specific anticarbohydrate antibody in these participants is attributable to a decreased number of Memory B cells (a subgroup of B lymphocytes in charge of T-independent responses). Traditional vaccinations, which are given to splenectomized patients to protect them from being infected by encapsulated organisms, can only act in the presence of both the spleen and its functioning marginal zone. As a result, the study will look at the level of memory B cells in the blood after three months and 1-year post-splenectomy.

Aim of the study: The objective of the study was to observe the short- and long-term effects of splenectomy on memory B cells in children.

Methods: This prospective case-control study was conducted at the Pediatric Surgery Department of Bangabandhu Sheikh Mujib Medical University, Bangladesh. The study duration was one year, from July 2015 to August 2016. A total of 26 children were selected through a purposive sampling technique for this study, where the control group consisted of 10 children, and the case group consisted of 16 splenectomized children.

Result: Among the case group participants, 56.25% were from the oldest age group of 12-15 years, and 37.5% were from the age group of 8-11-years. Male prevalence was high in both the control and the case group. Beta thalassemia was the primary indication for splenectomy for 81.25% of case group patients. Mean B lymphocyte was 39700.2 in the control group, 3655.3 at the 3-month follow-up of case group participants, and 3381.7 for those who had follow-up 1-year after splenectomy. The mean amount of IgM memory B cells in the control group was 17.92%; at the 3-month follow-up of the case, it was 18.96%, and at the 1-year follow-up, it was 4.34%.

Conclusion: In post-splenectomy individuals, immunological constitutions in memory B cells do not support a T-independent response and, therefore, vaccination.

Keywords: Spleen, Splenectomy, B-cell, Lymphocytes

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Introduction

The spleen is a lymphoid organ in the human body. It is the largest lymphoid in the body. Its vast population of immune cells and its clever

architecture that allows for optimum monitoring and phagocytosis of circulating blood components play a significant role in pathogen protection.^{1,23} The spleen's microanatomy is structured in such a

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way that each provides an optimal environment for diverse functions, such as hematologic function and immunological function, respectively.⁴ The hematologic and immune functions of the spleen include the ingrowth of vascular channels, the influx of reticular cells and related byproducts to form a filtering network, the migration of cells from other organs such as the bone marrow and thymus, and the final maturation of transient and resident cell populations. Splenectomy is the surgical removal of the spleen. Splenectomy is most commonly performed to repair a burst spleen, which is generally caused by an abdominal injury. Splenectomy may also be done to treat various problems such as a painfully enlarged spleen (splenomegaly), some blood abnormalities, malignancies, infection, and noncancerous cysts or tumors.⁵ After splenectomy, patients are often at high risk for infection. In some studies, although the prevalence of sepsis in post-splenectomy patients is low, it has a significant fatality rate, particularly in children with hematological abnormalities.⁶⁻⁸ This increased risk of infection has been related to the spleen's lack of filtration and generation of anticarbohydrate antibodies. According to a recent study on such individuals, a lack of this particular anticarbohydrate antibody in these participants is linked to a decreased number of IgM memory B cells (IgM⁺IgD⁺CD27⁺ B cells).⁹ Studies also found that the density of IgM memory B cells in the peripheral blood increases over the existing, corresponding to the density of increasing splenic marginal zone B cells in charge of T-independent responses.¹⁰ However, another research found that the circulating IgM memory B cell population was already well grown and mutated in healthy infants at one year of age, a developmental period in which the splenic marginal zone is not well-differentiated and functioning.¹¹ Because circulating IgM memory B cells reflect functioning splenic tissue and are linked with a low risk of infection and OPSI, the measurement of IgM⁺IgD⁺CD27⁺ B cells might be utilized to distinguish between individuals with a high or low risk of infection following Splenectomy. Traditional vaccinations, which are given to splenectomies patients to protect them from being infected by encapsulated organisms,

can only act in the presence of both the spleen and its functioning marginal zone. So, the study is designed to observe the level of IgM memory B cells in the blood following 3-months and 1-year of splenectomy.

General Objective

- To observe the immunological consequence following Splenectomy in children.

Specific Objectives

- To estimate long-term changes in IgM memory B cell level in the peripheral circulation.

Materials and Methods

This prospective case-control study was conducted at the Pediatric Surgery Department of Bangabandhu Sheikh Mujib Medical University, Bangladesh. The study duration was one year, from July 2015 to August 2016. A consecutive sampling technique was used to select the study participants from the children admitted at the pediatric surgery department of the study hospital for splenectomy for any causes. A total of 26 patients were selected for the study due to the short study period. The study participants were divided into two groups, the case group containing 16 patients and the control group consisting of 10 participants. Informed written consent was obtained from the legal guardians of each participant. Ethical approval was obtained from the study hospital's ethical review committee. The observational data sheet was filled by observation of blood levels and various serum levels.

Inclusion Criteria

- Children aged 2-15 years
- Patients whose parents had given consent to participate in the study.
- **Case:** Children who had their Splenectomy for any cause
- **Control:** Children admitted for any surgical procedures other than Splenectomy.

Exclusion Criteria

- Post Tonsillectomy child.
- Unwilling to participate.
- Exclude those affected with other chronic diseases or otherwise immunocompromised etc.

Results

Among the case group participants, 56.25% were from the oldest age group of 12-15 years, and 37.5% were from the age group of 8-11-years. Male prevalence was high in both the control and the case group. Beta thalassemia was the primary indication for splenectomy for 81.25% of case group patients. Mean B lymphocyte was 39700.2 in the control group, 3655.3 at the 3-month follow-up of case group participants, and 3381.7 for those who had follow-up 1-year after splenectomy. The mean amount of IgM memory B cells in the control group was 17.92%; at the 3-month follow-up of the case, it was 18.96%, and at the 1-year follow-up, it was 4.34%.

Table 1: Age distribution of the participants (n=26)

Age group (in years)	Control, n=10	Case, n=16
4-7	3(30%)	1(6.25%)
8-11	4(40%)	6(37.5%)
12-15	3(30%)	9(56.25%)

Among the control group participants, 30% were between the age of 4-7 years, 40% were between 8-11-years of age, and 30% were between the age of 12-15 years. In the case group, the majority (56.25%) were from the oldest age group of 12-15 years. On the other hand, 37.5% were from the age group of 8-11-years, and only 1 participant was from the youngest age group of 4-7 years.

Table 2: Gender distribution of the participants (n=26)

Gender	Control, n=10	Case, n=16
Male	7 (70%)	10 (62.50%)
Female	3 (30%)	6 (37.50%)

Among the study participants, the male prevalence was higher. In the control group, 70% were male, and 30% were female. In the case group, 62.5% were male, while the remaining 6 (37.5%) were female.

Table 3: Indication of splenectomy in the case group participants (n=16)

Indication of Splenectomy (n=16)	Frequency	Percentage
Beta Thalassemia	13	81.25%
Portal Hypertension	3	18.75%

For the 16 case group children, beta-thalassemia indicated splenectomy for 81.25% of cases. For the remaining portal hypertension, 3 participants were the indication for splenectomy.

Table 4: Level of B lymphocytes and IgM memory B cells in peripheral blood of control group subjects (n=10)

Variables	B lymphocytes (CD 19+)	IgM memory B cell	Percentage of Mean IgM memory B cell
Range	9642-72258	1669-17558	17.92%
Mean	39700.2	7716.2	

IgM memory B cell level range is (1669-17558), and the mean is 7716.2 in the healthy control group, and it constitutes about 18% of total B lymphocytes (Average of B lymphocytes is 39700.2).

Table 5: Level of B lymphocytes and IgM memory B cells in peripheral blood of case subjects (n=16)

Follow-up of Case participants		B lymphocytes (CD 19+)	IgM memory B cell	Percentage of Mean IgM memory B cell in B Lymphocytes
3-month follow-up (n=10)	Range	1100-7205	297-1327	18.96%
	Mean	3655.3	693.2	
6 month follow-up (n=6)	Range	1258-5436	58-284	4.34%
	Mean	3381.67	146.83	

Though the total count of B lymphocytes has decreased, i.e., Ave. the count is 3655.3, the percentage of IgM memory B cells remains almost the same (18.96%) in comparison to control subjects (17.92%) following 3-months of splenectomy. But the average B lymphocyte count following a 1-year splenectomy is 3381.67, almost similar to the 3-month count (3655.3). Still, the average count of IgM memory B cell level (146.83) and percentage of IgM memory B cell within B lymphocyte (4.34%) are notably reduced compared to the control group and the 3-month follow-up group.

Table 6: Comparison of Mean IgM Memory B cells of control and case groups

IgM Memory B Cells	Control (n=10)	Case (n=16)	
		3-month follow-up (n=10)	1-year follow-up (n=6)
Mean value	7716.2	693.2	146.8
Significance	n/a	<0.05	<0.05

The difference between the mean IgM memory cells of the control group and the case group, the 3-month follow-up (n=10), and the 1-year follow-up (n=6) were statistically significant.

Discussion

Many studies have been conducted to resolve the debate over whether splenectomized patients will respond to traditional pneumococcal vaccination after splenectomy. Some of them were product-based, nine while others were process-based.¹⁰⁻¹²

The present study was conducted on the process premise. The IgM memory B cell is a processing unit that the body uses to respond to an encapsulated bacterial antigen and the following immunization via the T-independent route. Dr. Kruetzmann researched three populations in 2003: splenectomized and asplenic patients, healthy

controls, and patients with common variable immunodeficiency (CVID). They evaluated the frequency of B cells in terms of mature B cells, switched memory, and IgM memory B cells in the blood of 14 patients who had been splenectomized six months to 9 years ago. There was a significant difference in the frequency of memory B cells between the healthy control group and the splenectomized patients.¹⁰ When the IgM memory B cell level in three asplenic infants was compared to that of their age-matched controls, it was discovered that it was just 0.6% in 13-month-old and 7-year-old children, whereas it was 15% and 11% in control patients. However, the 8-month-old infant had about the same frequency (0.2 percent) as the control (0.1%). It was also discovered that in CVID patients with a normal functional spleen who were not infected, the frequency of B cells and the IgM memory B cell population were normal. However, in CVID patients who had multiple infections, the IgM memory B cell population in their peripheral blood was undetectable. In our study, most of the case group participants were from the older age group, and only 1 participant was from the age group of 4-7 years. Male prevalence was high in the study's case and the control group. Of the case group participants, 62.50% were male, and the remaining 37.50% were female. The primary indication for splenectomy was beta thalassemia for 13 out of 16 case patients, and for the remaining three, the main cause was Portal Hypertension. Portal hypertension is caused by elevated pressure in the portal venous system, exceeding 5 mm Hg or portal vein to hepatic vein gradient

of greater than 10 mm Hg; variceal bleeding is associated with a gradient above 12 mm Hg.^{13,14} The primary indications of the present study were different from the findings of another 2008 study, where among 26 splenectomized patients, 19 had immune thrombocytopenic purpura (ITP), one had autoimmune hemolytic anemia and six had hereditary spherocytosis (HS).⁹ A 2013 study with a group of asplenic individuals (57 adults and 21 children) without additional immunologic defects found that spleen removal had diminished the frequency of total memory and IgM memory B

cells, confirming the important role of the spleen in the generation and maintenance of the IgM memory pool.¹⁵ It was also shown that, compared to adults, asplenic children had significantly fewer total IgM-secreting cells. Anti-pneumococcal IgM-secreting cells were considerably fewer in splenectomized youngsters than in healthy children. They found that the frequency of IgG and IgM memory B cells, as well as serum IgG specific for PnPS (Pneumococcal Polysaccharide), was related to the vaccination history of the children in their research. PCV (Protein conjugate vaccine) inoculation significantly raised anti-pneumococcal IgM-specific B cells in children. In this study, the mean value of Ig M memory B cell levels in control and splenectomized subjects, following 3-months and 1-year of splenectomy, were 7716.2, 693.2, and 146.83, respectively. So, in this age range study, the Ig M memory B cell levels depict that they were drastically reduced in peripheral blood of splenectomized patients about ten times and 50 times following 3-months and one year of splenectomy. Although this is not a cohort study, the result of 1-year following splenectomy patient displays proposed extra splenic sites are not sufficient enough to maintain the pool of Ig M memory B cells in peripheral blood. As a result, the level of these cells remains below a critical level for maintaining the expected T-independent response against antigens of the encapsulated organism. So, this study supports the previous studies more certainly by observing the antibody processing unit (Ig M memory B cell) for the proper performance of a T-independent response. A cohort design may appear to have been a better method to study the phenomena. Nevertheless, a case-control study as this has been designed does not suffer from serious setbacks from concluding as in cohort studies. In distinction from other studies, which were done on a mixed population of adults and children, this study is done solely on the pediatric age group with the inclusion of both vaccinated and non-vaccinated subjects. In the context of Bangladesh, we have utilized the optimum technology (Immunoflowcytometry) for such immunological study. Both the groups under study were strictly scrutinized for otherwise intact immunological

status, i.e., freedom from chemotherapeutic or immunosuppressive drugs and tonsillectomy or any other immunosuppressive autoimmune disease.

Limitations of The Study

The study was conducted in a single hospital with small sample size. So, the results may not represent the whole community.

Conclusion

In post-splenectomy individuals, immunological constitutions regarding memory B cells do not support a T-independent response and, therefore, vaccination.

Conflict of interest: None declared

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