Effects of Surgical Stress on Early Nonspecific Immune Response in Children

P. Santosh Prabhu · S. Sridharan · S. Ramesh

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Abstract Surgery alters the body's homeostatic balance and defense mechanisms. In adults transient postoperative cellular and humoral immunosuppression after different degrees of operative stress has been reported. In children the immunologic consequences of operations are not elaborated. This study investigates the effect of minor and major surgery on early nonspecific immune response in terms of neutrophil counts and function. Forty-three children undergoing minor and major elective procedures were studied. Blood samples were collected before, immediately after, and 72 h after surgery. Total white cell count, differential neutrophil count, and neutrophil phagocytic function were studied using nitroblue tetrazolium test. Children were divided into two groups—group 1 underwent minor surgery and group 2 major surgery. In group 1 there was a significant drop in total counts after surgery, but in group 2 total counts were not affected. In both groups, the percentage of neutrophils increased immediately after surgery but fell to near or less than preoperative levels 72 h after surgery. However, the assessment of neutrophil functions by nitroblue tetrazolium test in both unstimulated and stimulated forms revealed it to be unchanged in group 1. In group 2 the unstimulated

neutrophil function was elevated 72 h after surgery, whereas stimulated function was elevated immediately after surgery. Minor surgery does not alter the early nonspecific immune response. However, major surgery seems to induce a transient increase in neutrophil phagocytic activity.

Keywords Neutrophil phagocytic function · Nitroblue tetrazolium

Introduction

Infections in surgical patients are a complex problem related not only to virulence of the offending organism but also to a multitude of factors influencing the host's defense mechanisms. All forms of tissue injury, including planned operation, alter the body's homeostatic balance and defense mechanisms. While an immunocompetent patient's body is highly resistant to infections, sepsis is a major cause of morbidity and mortality in surgical patients.

In adults the alterations produced by operative procedures on metabolic and immune systems have been studied. Transient postoperative cellular and humoral immunosuppression after different degrees of operative stress has been reported [1–3].

In the pediatric population, the metabolic and systemic response to operative stress is not well understood and the immunologic consequences of acute injury are not known.

Host resistance has three functional elements:

1. External barriers such as intact skin and mucous membrane.
2. Nonspecific response consisting of inflammation, chemotaxis, opsonization, and phagocytosis. The target is coated by an opsonin creating a complex that binds to receptors on neutrophils and macrophages. These cells

P. S. Prabhu (✉)
Department of Pediatric Surgery, Kasturba Medical College,
Manipal 576104 Karnataka, India
e-mail: psantoshprabhu@gmail.com

S. Sridharan
Department of Pediatric Surgery,
Kanchi Kamakoti CHILDS Trust Hospital,
Chennai, India

S. Ramesh
Department of Anaesthesiology,
Kanchi Kamakoti CHILDS Trust Hospital,
Chennai, India

consume the complex to create a phagosome to which lysosomes attach. Lysosomes then release enzymes that kill the organism.

3. Specific response which responds to a specific antigen and requires the system to be previously primed by the antigen.

The process of inflammation serves to bring leucocytes to the site of microbial invasion, and phagocytosis of the organism by neutrophils has a significant role in ensuring uneventful postoperative period and favorable outcome. This study investigates the effect of clean and clean-contaminated surgery on function of neutrophils, comparing the function before surgery and in the early postoperative period.

Aims and Objectives

To elaborate and compare the effects of minor and major surgery on early nonspecific immune response and to study the correlation of these effects, if any, on the eventual outcome with regard to development of infection.

Materials and Methods

Forty-three children undergoing elective surgery at Kanchi Kamakoti Childs Trust Hospital, Chennai, were included in this prospective study done over a period of 2 years after obtaining approval by the Hospital Research Ethics Committee. All operations were either clean or clean contaminated. These children were divided into two groups:

- Group 1 Children undergoing minor surgery (duration <90 min, no extensive dissection, postoperative stay not more than 24 h). Eighteen children underwent minor surgery: circumcision ($n=5$), unilateral inguinal herniotomy ($n=4$), inguinal herniotomy and circumcision ($n=3$), excision of upper lip cyst ($n=1$), unilateral orchiopexy for palpable testis ($n=4$), and unilateral orchiopexy and circumcision ($n=1$).

Anesthesia: Induction with thiopentone, ketamine, single-dose caudal epidural with bupivacaine, nitrous oxide with oxygen and isoflurane by the mask. Nonsteroidal anti-inflammatory drugs were administered for postoperative analgesia.

Antibiotic prophylaxis with combination of amoxycillin with clavulanic acid is given intravenously.

- Group 2 Children undergoing major surgery (duration of surgery >90 min and/or intraperitoneal/extensive

extraperitoneal dissection, postoperative stay >24 h). Twenty-five children underwent major surgery: laparotomies—choledochal cyst excision ($n=2$), interval appendectomy ($n=4$), colostomy closure ($n=2$), ileostomy closure ($n=1$), Soave's pull-through ($n=2$), Ladd's procedure ($n=1$), excision of adrenocortical tumor ($n=1$), seromuscular biopsy and transverse colostomy ($n=1$); genitourinary surgeries—unilateral pyeloplasty ($n=3$), unilateral ureteric reimplantation ($n=2$), excision of bladder diverticula ($n=1$), right nephrectomy ($n=1$), clitoral reduction and vaginoplasty ($n=1$); and others—cleft palate repair ($n=3$).

Anesthesia: Induction with thiopentone and morphine. Vecuronium for relaxation, maintained with nitrous oxide, oxygen, and isoflurane through endotracheal ventilation and lumbar epidural with bupivacaine. Postoperative analgesia with intravenous morphine and/or continuous epidural bupivacaine for 48 h and/or nonsteroidal anti-inflammatory drugs depending on the nature of surgery.

Five of these children required packed cell transfusion during surgery.

Antibiotics: Third-generation Cephalosporins and aminoglycoside intravenously with added metronidazole for those with intestinal surgery starting at induction.

Investigations

Total white cell count, differential leukocyte count, platelet count and nitroblue tetrazolium (NBT) test—unstimulated and stimulated were done just before induction (S1), immediately after surgery (S2), and 72 h after surgery (S3). Venous blood samples of 2 ml were collected at these times after obtaining informed consent.

NBT Test When neutrophils are exposed to the yellow NBT dye, unstimulated neutrophils do not take up the dye. It is taken up by active neutrophils into their phagosomes and subjected to intracellular reduction which converts it into an insoluble blue-black crystalline form which is seen in light microscopy. Since the same intracellular reduction is essential for microbicidal activity, it is an indicator of neutrophil function.

Method Six drops of heparinized anticoagulated blood is mixed with six drops of working NBT solution. It is mixed gently and incubated at 37 °C for 30 min. Then smear is made and dried. Dry smear is stained with Leishman stain and examined under oil immersion. A total of 100 neutrophils are counted and the number of neutrophils with ingested dye

is noted as percentage. Simultaneously another smear is prepared with addition of *E. coli* endotoxin of 0.1 ml to the NBT solution. This stimulates neutrophils and more neutrophils take up the dye.

Interpretation In a healthy individual in the unstimulated state the number of active neutrophils may be as low as 10 %. On stimulation it may increase to as high as 90 %.

NBT test has been used in this study in both unstimulated and stimulated forms. S1 provides baseline neutrophil activity of the child prior to surgery, while S2 and S3 indicate the same immediately after and 72 h after surgery.

Statistical analysis was done using the Statistical Package for Social Scientists (SPSS) software version 10. Student's *t*-test was used for comparing the initial values. Subsequently the values were tested in three pairs using the paired *t*-test. *P* value less than 0.05 was considered significant.

Pair 1: S1 and S2

Pair 2: S1 and S3

Pair 3: S2 and S3

Results

There were 31 boys and 12 girls with the age range of 6 months to 13.5 years, the mean age being 60.83 months. Table 1 shows the patient characteristics in the two groups.

S1 values for total white cell count, differential neutrophil count, S1 NBT unstimulated and S1 NBT stimulated were compared between groups 1 and 2 using Student's *t*-test to ensure similarity and rule out differences in sampling. Once this was established each group was analyzed individually.

Results in Group 1

Total counts showed a significant drop immediately after surgery and 72 h after surgery, from a mean of 9,200 cells/

mm³ at S1 to 7,672 cells/mm³ at S2 and 7,061 cells/mm³ at S3. While the mean percentage of neutrophils increased from 46.72 % at S1 to 49.61 % at S2, the increase was not statistically significant. The same fell to 40.22 % at 72 h after surgery. There was no significant change in unstimulated neutrophil function with a small increase in activity from a mean of 60.72 % at S1 to 62.78 % at S2 and 62.27 % at S3. The same was the case with stimulated neutrophil function with an insignificant increase from 66.11 % at S1 to 68.06 % at S2 and 69.98 % at S3 (Table 2). None of these children had any clinical or laboratory evidence of sepsis or wound infection at 72 h and 10 days after surgery.

Results in Group 2

Total counts were similar before and after surgery—9,424 cells/mm³ at S1, 10,976 cells/mm³ at S2, and 9,828 cells/mm³ at S3. However, the percentage of neutrophils showed a significant increase from 48.92 % at S1 to 64.48 % at S2 but fell to 54 % at S3. Unstimulated neutrophil function increased immediately after surgery from 61 % at S1 to 68 % at S2 and remained elevated at 66.6 % at S3, a significant increase from the preoperative level. The stimulated neutrophil function also increased significantly from a mean of 67.8 % to 77.72 % at S2 and reduced to 72 % at S3. However, the difference between S1 and S3 was not significant (Table 3). None of these children had any clinical or laboratory evidence of sepsis or wound infection at 72 h and 10 days after surgery. Three had fever in the first 48 h which settled with paracetamol and did not require any change of antibiotic.

Comparison of the Two Groups

In group 1 there was a significant drop in total counts after surgery, but in group 2 total counts were not affected. In both groups the percentage of neutrophils increased immediately after surgery but fell to near or less than preoperative levels at S3. However, the assessment of neutrophil functions by NBT revealed an improvement in both unstimulated and stimulated functions in both groups though the increase was statistically significant only in group 2. But in both groups, at no point there was a reduction in neutrophil function below the preoperative level. None of the children in either group developed infection in the postoperative period.

Discussion

The immune system in humans is modulated by various forms of stress, infections, malignancies, nutritional status.

Table 1 Patient characteristics in the two groups

No.	Characteristics	Group 1	Group 2
1	Total No.	18	25
2	M:F	18:0	13:12
3	Age Range	1 year 11 months–10 years	6 months – 13 years 6 months
4	Mean age	68.7 months	55.1 months
5	Mean weight	18.25 kg	15 kg
6	Range of duration	20 – 90 min	40 – 275 min
7	Mean duration	49.44 min	147.6 min

Table 2 Comparison of neutrophil functions for the three pairs in group 1

Pairs	n	Unstimulated neutrophil function				Stimulated neutrophil function			
		Mean(%)	Standard deviation	Standard error of mean	p value for the pair	Mean(%)	Standard deviation	Standard error of mean	p value for the pair
S1	18	60.72	21.31	5.02	0.540	66.11	16.50	3.89	0.593
S2	18	62.78	17.76	4.19		68.06	17.83	4.20	
S1	18	60.72	21.31	5.02	0.621	66.11	16.50	3.89	0.350
S3	18	62.67	20.70	4.88		69.89	22.59	5.32	
S2	18	62.78	17.76	4.19	0.979	68.06	17.83	4.20	0.642
S3	18	62.67	20.70	4.88		69.89	22.59	5.32	

and trauma. Surgery has also been thought to alter the body's homeostatic balance and defense mechanism. The current knowledge obtained by studies on adult patients is complex, confusing, and not clearly defined. Surgery is believed to be an immunodepressant, at least transiently [1–3]. There are however reports contradictory to this, suggesting at least some parts of the immune response may be unchanged [4, 5] or enhanced [6]. The various cellular components, namely neutrophils, monocytes, and lymphocytes, have been studied [1, 3–18]. The phagocytic activity of neutrophils has been found to be reduced [3] or unchanged [4], while the serum opsonic activity is found to be decreased after surgery. Neutrophil chemotaxis is also reduced [4, 5]. An important factor predisposing to early postoperative infection may be a depressed neutrophil phagocytic activity and a lower serum opsonic activity after surgery. Interestingly aortic clamping seems to increase the respiratory burst activity of neutrophils, and these activated neutrophils have been shown to play a central role in the early stages of reperfusion injury [8]. Research work is also directed at agents which may act as biological response modifiers and improve immune response following surgery. Ranitidine [10] has been shown to improve neutrophil and monocyte function.

Immune response to operative stress in children is unclear. Early studies by Mollit et al. [11] showed that in children undergoing minor surgery the neutrophil phagocytosis,

random migration, killing, and chemotaxis were unchanged by operation. There was a significant neutrophil leucocytosis but decrease in absolute lymphocyte count. They conclude that these represent an appropriate mobilization of child's immune response to the stress of surgery with no evidence of functional depression. Romeo et al. [16] studied respiratory and phagocytic burst activities of neutrophils and monocytes in children undergoing surgery. They found these activities to be increased at the end of minor surgery in children with monocytic activity persisting 72 h after surgery. Merry et al. [12] found that the stress of major surgery does not alter the neutrophil chemotaxis and actin polymerization of older children.

This study shows that while minor surgery seems to bring about a significant reduction in total counts immediately after and 72 h after surgery, the percentage of neutrophils and phagocytic activity of neutrophils as assessed by NBT test are unaffected. Hence, minor surgery does not seem to alter the early nonspecific immune response much. The reduction in total counts does not seem to affect the outcome as none of the children developed infection. Major surgery does not alter the total counts but induces a significant increase in neutrophil population. It produces a significant increase in stimulated neutrophil function at the end of surgery, indicating that the neutrophils are in a heightened state of alertness and a higher number of inactive neutrophils are recruited in response to a challenge when compared to the preoperative status. The unstimulated function remains significantly elevated even

Table 3 Comparison of neutrophil functions for the three pairs in group 2

Pairs	n	Unstimulated neutrophil function				Stimulated neutrophil function			
		Mean(%)	Standard deviation	Standard error of mean	p value for the pair	Mean(%)	Standard deviation	Standard error of mean	p value for the pair
S1	25	61.66	17.17	3.43	0.085	67.80	19.21	3.84	0.000
S2	25	68.00	20.87	4.17		77.72	17.78	3.56	
S1	25	61.66	17.17	3.43	0.040	67.80	19.21	3.84	0.197
S3	25	66.60	18.18	3.64		72.52	18.25	3.65	
S2	25	68.00	20.87	4.17	0.706	77.72	17.78	3.56	0.174
S3	25	66.60	18.18	3.64		72.52	18.25	3.65	

after 72 h. It is hence clear that neither type of surgery is a depressant of the early nonspecific immune response, and this was reflected by the uneventful postoperative periods in these children with regard to development of infection. Interestingly, opening of peritoneum has been shown to reduce phagocytic activity in animal experiments [19]. However in our study, in group 2, the total count, percentage of neutrophils, and unstimulated neutrophil function showed identical changes in the laparotomy and nonlaparotomy surgeries. The stimulated neutrophil function was elevated above preoperative levels at 72 h after surgery in the nonlaparotomies, whereas in the laparotomies it returned to but did not fall below preoperative levels. However, these values and their role in outcome could not be compared statistically because of small sample size in each subgroup.

Our understanding of the effect of surgery on components of immune response is limited. This study has attempted to investigate the effect of surgery on early nonspecific immune response, namely neutrophil functions, which is the body's first line of defense, using simple hematology laboratory studies. Now, individual components of neutrophil functions such as chemotaxis, respiratory, and phagocytic burst activity can be measured using newer and more sensitive modalities such as flow cytometry. However, these are not easily accessible and expensive. Also, comprehensive investigations are required to study the effect of surgery on other components of the immune system.

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