

determining the time taken for repair and assessment of immune status is meaningful in such cases.

Since surgical trauma is followed by tissue repair that may take variable time to complete and phagocytosis is closely related to the repair process, the phagocytic function test is taken as a parameter to assess the nonspecific immunity and thereby the repair process following surgical stress.

Studies done to examine the effect of surgical stress on immune response in the past have shown contradictory findings (3–5). The present study was designed to elucidate whether immune response is altered following surgical stress. Polymorphonuclear leucocytes (PMN) play crucial roles in protecting the host against invading microbes (6). Neutrophils form the frontline of immunological defense and phagocytosis forms the main defense against infection (7). Hence the neutrophil phagocytosis is taken as a parameter to measure the non-specific immune response prior to and immediately after surgical stress.

MATERIAL AND METHODS

Patients admitted for elective surgeries under General Surgery Department, Kasturba Medical College Hospital, Manipal were selected as subjects. Consent from the college ethics committee and written consent from subjects were taken prior to the study. Males (17) and females (3) with mean age 43.4(±2) yrs were taken as subjects.

Criteria for patient selection : Adults of both sex posted for elective surgery (hernia repair)

were taken as subjects. These patients had no signs of acute infections (Normal preoperative total leucocyte count in peripheral blood), none of them were on any hormonal therapy.

Collection of the blood sample : Venous blood was collected; One sample on the day of admission (around 4–6 days before surgery) and second sample one day after surgery (postoperatively). The blood sample was collected in heparinised sterile syringes with all aseptic precautions, transferred to sterile test tubes and taken immediately for evaluation.

Investigative procedures : Candida phagocytosis by neutrophils in preoperative and post operative blood samples was performed according to the method described elsewhere (8) and as described below :

Neutrophils in the peripheral blood ingest heat killed *Candida albicans* in vitro, when optimum conditions are provided in a medium. The rate at which the *Candida* are ingested depends on the number of *Candida* in the suspension, pH of the medium and time of incubation but largely on the membrane integrity. After 30 minutes of incubation at 37°C in a suitable medium, normal neutrophils may contain anywhere from 0 to 4 *Candida*/cell.

Preparation of heat killed *Candida* suspension :

Candida is grown in a suitable medium like Saboraud's 2% dextrose medium for 48 hours at 37°C to obtain organisms in yeast form. These colonies are taken by means of a sterile loop and mixed with phosphate buffer saline. This is boiled for 15 min and

then centrifuged at $704 \times g$ for 10 min. Deposits are washed with PBS and stored at 4°C . The heat killed *Candida* suspension was prepared and counted in Improved Neubauer's chamber and optimum amount of the suspension required for the procedure was standardized (10^7 yeasts/ml).

Candida phagocytosis : Heparinised blood was collected from patients and mixed with pooled sera, *Candida* suspension and Hank's medium. The sample was centrifuged at $489 \times g$ for 10 min and plasma discarded. Buffy coat was aspirated by means of Pasteur pipette and transferred to test tube. The tube was kept in a water bath for incubation at 37°C for 30 min. The test tube was centrifuged at $176 \times g$ for 5–10 min. Clear supernatant solution discarded and buffy coat aspirated and taken on glass slides. Smears of this deposit were prepared.

Slides with the smear were fixed with methanol for 1 min and Lieshman's stain was added to cover the smear. After 1–2 min double the quantity of distilled water was added to the stain and the stain was blown frequently for uniform mixing. After 15–20 min of staining slides were washed under running water and dried. Stained smears were examined under oil immersion for the presence of *Candida* inside the neutrophils.

The number of neutrophils positive for *Candida* for 100 neutrophils gives the 'Phagocytic index'. Average number of *Candida* inside 100 positive neutrophils gives the 'Avidity index'. Percentage of neutrophils is found by differential count of the stained smear of peripheral blood sample. Samples were examined within six hours of drawing

blood from the patients. Out of 25 samples obtained, 3 samples were spoilt due to electricity problem and 2 samples were spoilt due to delay in conducting the procedure from the time of arrival of the sample. Thus phagocytic indices, avidity indices and differential count of neutrophils could be determined in preoperative and postoperative blood samples of 20 patients. Paired t-test was used and analysis was done using SPSS software.

RESULTS

Tables I–III give the results for the phagocytic index, avidity index and differential neutrophil count from 20 samples, respectively. The results revealed that the phagocytic index was significantly ($P=0.0001$) decreased in early postoperative period. The avidity index was however not significantly altered and the differential count of neutrophils was significantly increased during the early postoperative period.

TABLE I: Phagocytic index.

	<i>Preoperative</i>	<i>Postoperative</i>
Mean	51.5	42.1*
SD	± 4.02	± 6.79
SEM	± 0.9	± 1.52

* $P=0.0001$

TABLE II: Avidity index.

	<i>Preoperative (%)</i>	<i>Postoperative (%)</i>
Mean	1.133	1.113*
SD	± 0.09	± 0.08
SEM	± 0.02	± 0.02

* $P=0.52$

TABLE III: Differential count of neutrophils.

	<i>Preoperative (%)</i>	<i>Postoperative (%)</i>
Mean	62.3	68.5*
SD	±6.2	±9.03
SEM	±1.39	±2.02

*P=0.003

DISCUSSION

In the present study, the patients were exposed to elective operative procedure which is a form of stress. We observed a decrease in the phagocytic index meaning that the percentage of neutrophils involved in phagocytic activity was decreased by surgical stress. It probably involved the influence of the hormone cortisol which is known to depress the non-specific immune response. The other pathways which have been implicated in modulation of immune system by stress namely autonomic nervous system (9, 10), neuropeptides (11) and neurotransmitters (12) might also be involved. However, the avidity index was not altered significantly by surgical stress. This means that the phagocytic capacity of individual neutrophils was not affected by surgical stress in the present study. This finding suggests that there was no immunosuppression at the cellular level.

This finding has very significant implications on various aspects of peri and post operative surgical care and the tissue

repair in the postoperative period. The capacity to control these responses in surgical patients may have important clinical applications (13).

The immunosuppression in the early postoperative period following surgical stress that was observed in our study could be prolonged under several conditions, leading to postoperative complications (14). To assess the relation of the magnitude of surgical stress to repair process, further studies have to be done to assess the immune status not only in the early postoperative period but also on successive days until recovery. Simultaneous assessment of the hormonal status of the person during the postoperative recovery and also noting the postoperative care given to the patients (receiving antibiotics, immuno-nutrition therapy if any) have to be undertaken in future study. This would enable us to relate the effect of postoperative care on overcoming the initial immunosuppression and building up the phase of anabolism to bring about uneventful postoperative recovery (13).

In the light of present study, it could be concluded that surgical trauma is a form of stress which causes suppression of the immediate non-specific immune response. The surgical stress causes an overall decrease in the immune response of neutrophils whereas the phagocytic capacity of the individual neutrophils have been retained.

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