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Immune Responses to General Anaesthesia with Endotracheal Intubation and Spinal Anaesthesia in Patients Undergoing Elective Surgery in Korle-Bu Teaching Hospital ACCRA, Ghana: A Baseline Study

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Abstract: ***Surgical operations and anaesthesia have been shown to cause a variety of immunological disturbances in patients. The implications of these observations are that host defenses may be compromised by surgical and anaesthesia procedures. Study participants of American Society of Anaesthiologists (ASA) physical status I or II undergoing surgery under general anaesthesia with endotracheal intubation (mastectomy, thyroidectomy) and spinal anaesthesia (herniorrhaphy) were recruited for the study. Blood samples were collected pre-operatively within 24 hrs before anaesthesia, after anaesthesia induction before incision and post- operatively at 24 hrs, and 48 hrs, from each of the study participant. Full blood count was done using automated full blood count machine and Fluorescence Activated Cell Sorting machine was used to analyse the CD3+ CD4+ and CD3+ CD8+ cells. The result of the study indicates suppression of the cellular immune responses up to 48 hours after surgery. Decreased in the cell mediated immune responses was found to be significant (P = 0.004) in general anaesthesia group as compared to the spinal anaesthesia group at 48 hours after surgery. The magnitude and duration of the reduction in cell numbers and the subpopulation affected was related to the degree of surgical trauma, stress, pain and the anesthetic agents used. Anaesthesia and surgery therefore suppresses the cellular immune responses up to 48 hours after surgery.***

Keywords: Immune Responses, airways, Anaesthesia, Surgery, Cellular immunity

1. Introduction

All perioperative and postoperative periods are characterized by changes in the immune system. Tissue damage, anaesthesia, analgesia, stress and pain are the main modulators of immune system pre and post-operatively [1, 2].These reactions, depending on their severity, can have variable effects, ranging from the patient's susceptibility to infection, wound healing, systemic inflammatory responses, and multiple organ failure[2]. Anaesthesia plays a very important role in postoperative outcomes. Studies have shown that deep anaesthesia may be associated with adverse outcomes including mortality[1,3].Volatile anaesthetics was found to exert immunomodulatory effects by affecting endothelial expression of adhesion molecules and the secretion of cytokines and chemokines which enhances inflammation [4,5,6]. The intensity of such an inflammatory response is dependent on many factors, including the magnitude of tissue damage, the patient's pre-existing diseases, the type of surgery and surgeon's experience, as well as the anaesthesia regimen[7,8]. Moreover, drugs employed for inducing and maintaining general anaesthesia such as opioids and muscle relaxants as well as sevoflurane were also found to exhibit a pro-apoptotic effect on lymphocyte cells by decreasing mitochondrial transmembrane potential or activating extrinsic cell death pathways [7]. These could account for some unexplained post-surgical infections and complications observed in KBTH. These result in prolong hospital stay with significant economic impact on the patients. Risk factors associated with the likelihood of developing post-surgical infections are influenced by a number of factors which include patient's factors such as the immune system, anaesthetic factors, and surgeon experience. However, there is no documented information or data on the effect of anaesthesia and surgery on systemic immune responses following general aneasthesia with endotracheal intubation and spinal anaesthesia after surgery at KBTH .In this study systemic immune responses to general anaesthesia with endotracheal intubation and spinal anaesthesia in patients undergoing elective surgery (mastectomy, herniorrhaphy and thyroidectomy) was investigated.

2. Methodology

This was a prospective cross-sectional study of 17 males (50 ± 14 years) and 20 females (44±14 years) of American Society of Anaesthiologists (ASA) physical status I or II undergoing surgery under general anaesthesia with endotracheal intubation (mastectomy, thyroidectomy) and spinal anaesthesia (herniorrhaphy). The study was approved by the Ethical and Protocol Review Committee of the University of Ghana Medical School.

2.1 Preoperative Preparation

After an explanation of the study and following informed consent, preoperative clinical assessment was done for all the study participant within 24 hours in the wards before the surgery to exclude those on immunosuppressive drugs, radiotherapy, blood transfusion, chemotherapy, corticosteroids or with any co-morbid condition such as lymphomas and immunodeficiency considered generally as a risk factor for the study under investigation. Food was withheld from the study participant for 8-12 hours to minimize the risk of vomiting and regurgitation during anaesthesia and fluids were withheld for 2-3 hours before surgery. Midazolam (7.5-15mg) was given orally in the night and in the morning before surgery to calm or sedate the study participant. All patients were infused with 0.5-1 liter of normal saline before the start of the surgery. However the base line sample was taken after pre-medication and before infusion within 24 hours before the start of the surgery.

2.2 Anaesthesia Induction

General anaesthesia was induced with intravenous propofol (1.5-3 mg/kg) and pethidine (0.5-1mg/kg), morphine (0.05-0.1 mg/kg) or fentanyl (0.5-1µg/kg) analgesic , followed by vecuronium (0.1 mg/kg). General anaesthesia was maintained with 0.5%-2.0% isoflourane or halothane. Intravenous propofol and opioids were used as required to maintain an appropriate anaesthetic depth for the surgery. Airway was secured by an endotracheal tube and they were mechanically ventilated. At the last skin suture the inhalational anaesthetic agent was discontinued and participants that received general anaesthesia were fully conscious before they were extubated. Spinal anaesthesia was induced with 2-3 ml of 0.5% heavy bupivacaine after skin infiltration with 2ml of 1% lidocaine. Muscle relaxation was reversed with neostigmine (0.05 mg/kg) and atropine (0.02 mg/kg) in all the study participants. Anaesthesia was maintained for at least 30 ± 5 minute (0.5 hours) for sample to be taken before the start of the surgical procedure**.** The patient's physiological state and depth of anaesthesia was monitored continuously. Heart rate, blood pressure and peripheral oxygen saturation were monitored and recorded at five minute intervals. Paracetamol (1g 6 hourly) or diclofenac (100mg twice daily) suppository with 1-1.5 mg/kg intramuscular pethidine was given as post-operative analgesic.

2.3 Sample Collection and Analysis

Three milliliters (3 ml) of peripheral blood sample was collected from an antecubital vein of the arm contralateral to the intravenous infusion in EDTA vacutainer tube as follows: pre-operatively within 24 hrs before (any infusion and) surgery and 30 ± 5 minute (0.5hours) after anaesthesia induction (before incision) and post- operatively at 24 hrs, and 48 hrs, from each of the study participant. The samples were transferred in to ice box and carried immediately to laboratory for analysis. Samples were analysed within 3-8 hrs of collection**.**Full blood count was done using automated full blood count machine (Cell Dyn 3700, ABBOTT LABS, and USA). Four colour FACS **(**fluorescence activated cell sorting) lyse no-wash analysis was performed using FACSCalibur E975006436 (Becton, Dickinson and Company, United States) to analyse the $CD3^+$ $CD4^+$, and $CD3^+$ $CD8^+$ positive cells. Twenty microliters (20µl) of multi test reagents containing monoclonal antibody (CD3, CD4, and CD8) was vortexed and was pipetted in to labeled trucount tubes. Fifty microliters (50µl) of whole blood was added to each trucount tube. The tubes were votexed and incubated in the dark at 25°C for 15 minute. After incubation 450µl of FACS lysing solution was added and the tubes were votexed and re-incubated in the dark at room temperature. After incubation, samples were analyzed using the FACSCALIBUR.

3. Statistical Analysis

Statistical analysis of the data was performed using the Statistical Package for the Social Sciences (SPSS), version 20.0 (SPSS, Inc., Chicago, IL, USA). Values are expressed as the mean ± standard error of the mean. Friedman's test was used to assess differences in the cell count over time within the surgical groups. Krukal Wallis test was used to assess the differences in the clinical characteristics and cell count between the surgical groups under study. Statistical significance was considered for p values less than 0.05.

4. Result

The clinical characteristics of the surgical groups studied are summarized in Table 1.There were no significant differences between the surgical groups in terms of age (P = 0.8), weight (P = 0.6), time from induction to skin incision (P = 0.9). However there was a significant difference in terms of time from skin incision to the end of surgery (P = 0.04). The number of all circulating leukocyte populations decline in all the surgical groups understudy at 0.5 hr following anesthesia administration and persist up to 48hr after surgery except basophils in mastectomy, monocyte and eosinophils counts in thyroidectomy group which increases by 100%, 133% and 19% respectively at 48hr after surgery as compared to the baseline value. Neutrophils count declined significantly(P = 0.02) by 71.07% in the spinal anaesthesia (Herniorrhaphy) group whiles in the general anaesthesia group CD 8+ cells count declined significantly (mastectomy; P= 0.04, -62.45%, thyroidectomy; P = 0.02, -56%,) at 48hours after surgery as compared to the baseline values. The decreased in the CD 8+ cells count was observed to be significant (P = 0.004) in general anaesthesia group as compared to the spinal anaesthesia group at 48 hours after surgery (thyroidectomy; 197.5 ± 93.5(SEM), mastectomy; 184.6±86.9 (SEM), Herniorrhaphy; 480.2±89.7 (SEM). CD4+ cell count at 48hours post-surgery was observed to be almost equal to the baseline count in spinal anaesthesia group [baseline; herniorrhaphy 795.10±126 (SEM), 48 hours; herniorrhaphy 806.20 ± 118.00 (SEM)] whiles in general anaesthesia group, it declined up to 48 hours post-surgery (thyroidectomy ; -39.10% , mastectomy; -47.87%). However no significant difference was observed between the baseline count and the count at 48 hours [thyroidectomy (P = 0.3), mastectomy (P = 0.16), herniorrhaphy (P = 0.94)]. Different pattern of responses was observed in the monocyte, eosinophils and basophils count in the entire surgical group under study.

Monocyte and eosinophils count declined in the mastectomy and herniorrhaphy group up to 48 hours after surgery whiles in the thyroidectomy group an increase was observed as compared to the baseline value. Basophils count decreased in the thyroidectomy and herniorrhaphy group whiles in the mastectomy group an increased was observed as shown in table 5, 6 and 7.

Table 1: Clinical Characteristics of the Surgical Groups

Surgery	*Thyroidectomy(GA)*	*Mastectomy(GA)*	*Herniorrhaphy(SP)*	*P- value*
Age/years	43.00 ± 14.80	45.00 ± 10 .70	50.00 ± 14.00	0.80
Weight /kg	66.90 ± 10.00	74.00 ± 16.00	60.00 ±11.00	0.60
M/F	3.00/9.00	0.00/12.00	11.00/2.00	
Time from induction to skin incision /minute	30.00± 5.00	30.00± 2.00	30.00 ± 3.00	0.90
Time from skin incision to end of surgery/ hour	2.00± 0.25	3.00 ± 0.33	1.50 ± 0.20	0.04
Normalsaline/litres before the start of surgery	1.00 ± 0.50	1.00 ± 0.50	1.00 ± 0.50	

Male (M), Female (F), Kilogram (Kg), Values are expressed as mean ± standard deviation.

Table 2: Mean CD8+ Cell Count /µLITER

Surgery	Baseline	0.5 hours	24 hours	48 hours	Per. change	P-value
Thyr(GA)	455.50±118	463 ±113	327 ±116	197.50±93.50	-56.64	0.02*
Mast(GA)	491.60±111	422.9±85.3	285.9 ± 79.4	184.60±86.90	-62.45	0.04*
Hem(SP)	699.00±110	644.8±78.4	539 ±75.3	480.20±89.7	-31.30	0.14

Mean CD8+ cell count /µLiter and percentage (Per.) change (%) at 48 hours in patient that undergo surgery under general anaesthesia(GA)(mastectomy, thyroidectomy) and spinal anaesthesia(SP) (herniorrhaphy). The results are expressed as the mean ± standard error of the mean (SEM). Reference range: 190.00-1140.00.

Table 3: Mean CD4+ Cell Count/µLiter

Surgery	*Baseline*	*0.5 hours*	*24 hours*	*48 hours*	*Per. change*	*P*
Thyr(GA)	741.40±177	701±151	485±178	451.50±213.00	-39.10	0.3
Mast(GA)	754.80±166	685±143	520±162	393.50±183.00	-47.87	0.16
Hem(SP)	795.10±126	760±106	792±113	806.20±118.00	1.39	0.94

Mean CD4+ cell count /µLiter and percentage (Per.) change (%) at 48 hours in patient that undergo surgery under general anaesthesia(GA)(mastectomy, thyroidectomy) and spinal anaesthesia(SP) (herniorrhaphy). The results are expressed as the mean ± standard error of the mean (SEM). Reference range: 410.00- 1590.00.

Table 4: Mean Neutrophils Count $X10^9$/Liter

Surgery	*Baseline*	*0.5 hours*	*24 hours*	*48 hours*	*Per. change*	*P*
Thyr (GA)	0.98 ± 0.75	0.77±0.26	0.94 ± 0.40	0.69 ± 0.34	-29.06±3.66	0.55
Mast(GA)	1.15± 0.52	0.749±0.27	0.32 ± 0.12	0.39 ± 0.34	-65.33±18.70	0.24
Her(SP)	1. 57 ± 0.39	1.297±0.49	1.29 ± 0.38	0.45± 0.00.22	-71.07±9.45	0.02*

Mean Neutrophils count $x10^9$/Liter and percentage (Per.) change in patient that undergo surgery under general anaesthesia(GA)(mastectomy(Mast), thyroidectomy(Thyr)) and spinal anaesthesia(SP) (herniorrhaphy(Hern) . The results are expressed as the mean ± standard error of the mean (SEM). Reference range: 2.00- 6.90

Table 5: Mean Monocyte Count $X10^9$/Liter

Surgery	Baseline	0.5 hours	24 hours	48 hours	Per. change	P
Thyr (GA)	0.03± 0.01	0.02±0.01	0.05± 0.03	0.07±0.02	133.33	0.39
Mast(GA)	0.14±0.06	0.18±0.16	0.35±0.20	0.03±0.01	-78.57	0.10
Her(SP)	0.04±0.01	0.24±0.14	0.10 ±0.10	0.02±0.02	-50.00	0.07

Mean Monocyte count $x10^9$/Liter and percentage (Per.) change in patient that undergo surgery under general anaesthesia(GA)(mastectomy(Mast), thyroidectomy(Thyr)) and spinal anaesthesia(SP) (herniorrhaphy(Hern) . The results are expressed as the mean ± standard error of the mean (SEM). Reference range: 0.00-0.90

Table 6: Mean Eosinophils Count $X10^9$/Liter

Surgery	Baseline	0.5 hours	24 hours	48 hours	Per. change	P
Thyr (GA)	0.43±0.2	0.46±0.16	0.53±0.09	0.51±0.29	18.61	0.83
Mast(GA)	0.32±0.12	0.62±0.27	0.21±0.08	0.09±0.04	-72.19	0.09
Her(SP)	0.27±0.05	0.31±0.17	0.37±0.15	0.10±0.04	-64.82	0.01*

Mean Eosinophils count x10^9/Liter and percentage (Per.) change in patient that undergo surgery under general anaesthesia(GA)(mastectomy(Mast), thyroidectomy(Thyr)) and spinal anaesthesia(SP) (herniorrhaphy(Hern). The results are expressed as the mean ± standard error of the mean. Reference range: 0.00- 0.70.

Table 7: Mean Basophils Count X 10^9/Liter

Surgery	Baselin	0.5 hours	24 hours	48 hours	Per. change	P
Thyr (GA)	0.003±0.002	0.007±0.003	0.004±0.002	0.002±0.001	-33.333	0.540
Mast(GA)	0.002±0.001	0.003±0.001	0.014±0.004	0.004±0.002	100.000	0.721
Her(SP)	0.015±0.020	0.001±0.001	0.021±0.007	0.009±0.004	-40.000	0.574

Mean Basophils count x10^9/Liter and percentage (Per.) change at 48 hours in patient that undergo surgery under general anaesthesia(GA)(mastectomy(Mast), thyroidectomy(Thyr)) and spinal anaesthesia(SP) (herniorrhaphy(Hern)). The results are expressed as the mean ± standard error of the mean (SEM). Reference range: 0.00 - 0.20.

Table 8: Leukocyte Count 48 Hours After Surgery

Cells	Thyr(GA)	Mast(GA)	Hern(SP)	P-value
Neutrophils	0.690 ± 0.340	0.390 ± 0.340	0.450 ± 0.002	0.273
Eosinophils	0.510±0.290	0.090 ±0.040	0.100 ± 0.040	0.594
Monocytes	0.070±0.020	0.030 ±0.010	0.020 ± 0.020	0.210
Basophils	0.002±0.001	0.004±0.002	0.009 ±0.004	0.230
CD4+	451.500±213.000	393.500 ±183.000	806.200±118.000	0.530
CD8+	197.500 ± 93.500	184.600±86.900	480.200±89.700	0.004

Mean Neutrophils, Monocytes, Eosinophils, Basophils cell count x10^9/Liter, CD4+ and CD8+ count /µLiter, at 48 hours post-surgery. The results are expressed as the mean ± standard error of the mean (SEM).

5. Discussion

In recent years, anaesthesia and surgery has gained attention with sufficient accumulation of knowledge and improvement of techniques in order to provide optimum surgical conditions for both the patient and the surgeon and to minimize morbidity and mortality after surgery. However, the immunologic effects of anaesthesia and surgery have been one of the less investigated areas in Ghana. The immunosuppressant effects of anaesthesia and the alteration of the immune function was considered as early as 1903 when Snel reported that ether, chloroform, and chloral hydrate increased the mortality from anthrax in guinea pigs [9] . In recent years, several reviews have revealed that anaesthesia and surgery can adversely affect both non-specific and specific components of the immune responses [8,10, 11, 12,13].In this study a declined in the number of all circulating leukocyte populations at 48 hours after surgery was observed except basophils in mastectomy, monocyte and eosinophils in thyroidectomy which increases slightly as compared to the baseline. Anaesthesia and surgery were found to depress the immune system by direct effects of anesthetic agents and indirect interaction of hormonal and metabolic changes caused by surgical stress[14]. It has been demonstrated that anaesthetic agents at clinical concentrations depress the functions of the immune responses by compromising inflammation, phagocytosis, lymphocyte transformation, cytotoxicity, antibody response to antigen and chemotactic functions of immune cells[13,14]. The immunopathologic consequences of chronic exposure to the inhalation anaesthetic agents was found to include down regulation of neutrophils and leucocytes cells[14] which is in agreement with the result obtained in this study. The different activation of T-helper (Th) cell subtypes induced by the stress of the surgery and the anaesthesia could also account for the significant decreased in $CD8^+$ cell count observed at 48 hours after surgery in this study. The principal role in this process may be played by glucocorticoids, which stimulate the production of cytokines that favour Th2 lymphocyte over Th1 lymphocyte[8, 15, 16] .Buggy *et al* , (1999) noted that there was a measurable decrease in the production of cytokines that favour the proliferation and differentiation of $CD8^+$ cells such as IL-2, IL-12, and IFN-γ, and an increase in the production of cytokines that interfere with the proliferation and differentiation of $CD8^+$ cells, such as IL-10. Although cytokines (IL-2, IL-12, and IFN-γ,), and glucocorticoid were not determined in this study, it is likely that interaction between glucocorticoid and T-lymphocyte subtypes might have played a role in the decreased $CD8^+$ levels up to 48 hours post- surgery.

Comparing the various surgical groups, mastectomy shows greater decreased in the lymphocyte count as compared to thyroidectomy and herniorrhaphy group. This could be due to the type of condition, anaesthetic technique and the surgery type in the various groups used for the study.

One could predict more susceptibility to infections in mastectomy group postoperatively. Close Postoperative follow up of the patients in the mastectomy group shows sepsis and delayed wound healing as compared to other surgical groups under study. The inhalational anaesthetic agents used and differences in the time from skin incision to the end of the surgery could account for the decreased in the leukocyte count observed in general anaesthesia group as compared to the spinal anaesthesia group at 48 hours after surgery. The general anaesthesia groups (mastectomy, thyroidectomy) were maintained on either halothane or isoflurene during surgery while the spinal anaesthesia group (herniorrhaphy) were not exposed to any inhalational anaesthetic agent. Several studies suggest that inhalational anaesthetics interfere with many phases of the immune

system: bone marrow depression, inhibition of phagocytosis and macrophage mobility [6,8,12,,17]. The migration of polymorphs, monocytes and other phagocytic cells is one of the earliest events in the body's defense against injury and infection. Study by Moudgil *et al.,* (1986), [18] revealed a dose-dependent depression of chemotactic migration by local, intravenous and inhalational anaesthetic agents. Hence the decreased in the leukocyte count observed in the general anaesthesia group as compared to the spinal anaesthesia group at 48 hours after surgery could be due to decreased in the chemotactic migration of the leukocyte as a result of exposure to the inhalational anaesthetic agents.

6. Conclusion

In conclusion, the study shows that major surgery and anaesthesia suppresses cellular immunity for several days. The decreased in the cell mediated immune responses was greater in general anaesthesia group (mastectomy, thyroidectomy respectively) as compared to the spinal anaesthesia group (herniorrhaphy). Spinal anaesthesia appears to cause less immunosuppression than general anaesthesia. Surgery is a necessary procedure, and provides a window of opportunity for the immune system to eliminate or gain control over minimal residual disease. However, surgery bears many risks which include perioperative psychological stress, Anaesthesia, pain, and trauma-related stress responses leading to immune suppression. It is clear that surgery and anaesthesia are necessary procedures and cannot be avoided; therefore pre and post-operative management and pain management may play a key role in influencing the immune responses in the postoperative period. This study provides a base line for further studies in the cellular immune responses to anaesthesia and surgery in Korle -Bu teaching hospital. Larger number of patients and different operative procedures and cytokines profile should be considered to ascertain the beneficial effects of immunomodulation in patients undergoing surgery.

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