Pre-, Intra- and Post-Operative Plasma Levels of Soluble P-Selectin in Diabetics under Thoracic Paravertebral Block versus General Anaesthesia

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Platelet activation that occur after tissue injury increases the expression of P-selectin. General anaesthesia and surgery may lead to peri-or post-operative hypercoagulability state that may lead to thrombotic complications, especially in high risk patients as diabetics. Administration of local anaesthesia was suggested to limit this hypercoagulability. The aim of this work was to evaluate the pre-, intra-and post-operative plasma levels of soluble P-selectin, as a predictor of thrombotic events, in diabetics receiving paravertebral block versus general anaesthesia during mastectomy operation for cancer breast. Forty type-2 diabetic females were included. They were randomly divided into two equal groups: group I received general anaesthesia and group II received thoracic paravertebral block. All females were subjected to preoperative thorough clinical examination, electrocardiography and laboratory investigations including complete blood picture, prothormbin activity, glycated hemoglobin A1c, fasting plasma levels of glucose, creatinine, lipid profile and alanine aminotransferase activity. Creatine kinase (CK), total and CK-MB, activities were also done preoperatively and six hours postoperatively. Plasma soluble(s) P-selectin levels were estimated preoperatively, 15 minutes after skin incision and one hour postoperatively. The results revealed that the preoperative plasma P-selectin levels did not significantly differ in the two groups. Its intra-and post-operative levels showed significantly higher levels in both groups than those preoperative, but the increase in group II is significantly less than those in group I. In conclusion, in high risk patients as diabetics undergoing major surgery, the use of paravertebral block is preferable as a good and effective alternative to general anaesthesia, to reduce the possibility of occurrence of thrombotic complications.

P-selectin (CD62P) is a member of the selectin family of cell adhesion molecules (Bevilacqua *et al.,* 1993). It is an integral membrane glycoprotein found on the external surface of both activated platelets and endothelium (Bevilacqua *et al.,* 1993; Blann *et al.,* 2003). Normally, the intact endothelium prevents platelet activation, but intimal injury associated with endothelial denudation and plaque rupture exposes sub-endothelial collagen and von Willebrand factor, supporting prompt platelet adhesion and activation (Huo & Xia, 2009; Man Fred *et al.,* 2010). Local platelet activation, then, promote the recruitment of platelets and the formation of thrombus (Huo & Xia, 2009; Man Fred *et al.,* 2010; Rinder *et al.,* 1991). After cellular activation by agoinst, e.g. thrombin, P-selectin is rapidly redistributed to the cell surface and binds a sialylated carbohydrate structure expressed on neutrophils and monocytes (Polley *et al.,* 1991; Kageyama *et al.,* 2007). Thus, P-selectin mediates platelet-leukocyte and endothelial cell-leukocyte adhesive interaction (Palabrica *et al.,* 1992; Furman et al., 2001). Soluble form of P-selectin is present in normal circulation and is increased in thrombotic disorders (Katayama *et al.,* 1993; Ay *et al.,* 2008).

Platelet activation is central to the formation of thrombosis which precipitates most unstable coronary syndromes (Rinder *et al.,* 1991; Freedman & Loscalzo, 2002; Jennings, 2009; Pabinger & Ay, 2009). A link between inflammation and thrombosis in acute coronary syndromes is the formation of

platelet-monocyte aggregates when platelets bind to surface-expressed P-selectin (Freedman & Loscalzo, 2002). Circulating platelet-monocyte aggregates have been shown to be an early marker of acute myocardial infarction, which is commonly diagnosed by measuring markers of cardiac necrosis that failed to provide direct information about platelet activation (Furman *et al.,* 2001). Thus, sP-selectin may serve as an early marker of platelet activation and thrombosis-induced impeding acute myocardial infarction (Ay *et al.,* 2008; Ikeda *et al.,* 1994; Hillis *et al.,* 2002).

Vascular thrombosis is a hallmark of type 2-diabetic patients (Colwell, 2001) and coronary thrombosis is the leading cause of death among them (Haffner *et al.,* 1998). A procoagulant or prothrombotic state had been found in these patients (Jokl & Colwell, 1997).

Surgical stress is associated with hypercoagulable and proinflammatory state that persists till the postoperative period (Lin *et al.,* 2009). Perioperative inflammatory responses to trauma can trigger the hypercoagulability, espeically in high risk patients who are liable to thromboembolic events such as diabetics (Mu Leudzi *et al.,* 2009; Ray *et al.,* 2010).

It was reported that volatile anaesthesia can produce increased expression of P-selection and release potent procoagulents (Frohlich, 1998), while local anaesthesia may be able to limit the perioperative hypercoagulability state (Langenecker, 2004).

The present study aimed to evaluate the pre-, intra-and post-operative plasma levels of s P-selectin, as a predictor of thrombotic events, in diabetics under thoracic paravertebral block versus general anaesthesia during mastectomy operation for cancer breast.

Subjects and Methods

Forty type-2 diabetic females were included after taking their consent. They were admitted to the Surgery Department of the Medical Research Institute Hospital, Alexandria University. Females with previous myocardial infarction, hypertension, dysrrhythmia, bronchial asthma, renal or hepatic insufficiency were excluded. Those receiving antiarrhythmic drugs, β-blockers, calcium channel blockers or angiotensin converting enzyme inhibitors were also excluded. In addition, females with coagulopathy, neuromuscular diseases, skin infection or apparent deformity in the vertebral column or those with difficult intubation or limited neck extension were excluded.

Half an hour before admission to the operating theater, all females were premedicated with midazolam 0.05 mg/kg intramuscularly and insertion of an intravenous cannula was done. For those receiving general anesthesia, 1.5 μg fentanly/kg was given intravenously in the operating room.

The forty females were randomly divided into two equal groups. Group I received general anaesthesia, where preoxygenation was carried out, followed by induction of 5 mg/ kg thiopental sodium, then 1mg/kg succinylcholine was administered followed by manual ventilation with 1% isoflurane in oxygen till complete neuromuscular blockade was achieved. Maintenance was done by isoflurane 1% and nitrous oxide 60% in oxygen. Incremental doses of atracurium after recovery from the depolarizing muscle blockade were used for mechanical ventilation. Then at the end of operation, 0.04 mg neostigmine/ kg and 0.01-0.02 mg atropine sulphate/ kg were given intravenously. Group II received paravertebral block at 4^{th} thoracic vertebra, using ropivacaine 0.75% in a dose of 1.5 mg/ kg through an epidural catheter followed by infusion of 0.25% ropivacaine at a rate of 10 ml/ hour. Sedation was also given (0.1mg propofol / kg/ min).

All females were subjected to preoperative investigations which included full history and thorough physical examination, and 12 lead resting electrocardiography. The preoperative laboratory tests included complete blood picture, prothrombin activity, glycated hemoglobin A_{1c} and estimation of fasting plasma levels of glucose, creatinine, cholesterol (total and LDL& HDL fractions) and triglycerides, as well as determination of alanine aminotransferase (ALT) activity (Burtis *et al.,* 2008). Plasma activities of both

total creatine kinase (CK) and CK-MB were determined (Burtis *et al.,* 2008) preoperatively and six hours postoperatively. Plasma sP-selectin levels were estimated (Kappelmayer *et al.,* 2004) preoperatively, 15 minutes after skin incision and one hour post operatively. All samples for s P-selectin estimation were stored in separate eppendorf tubes at-70ºc till the time of assay. It was quantitatively estimated by sandwich non-competitive enzyme linked immunosorbant assay kits (from Bender Medsystem Gm bH; Vienna. Austria), using biotin conjugated monoclonal anti-sP-selectin antibodies and streptavidin-horse raddish peroxidase and micro well plates coated with anti-sP-selectin monoclonal antibodies.

Continuous postoperative monitoring of heart rate, mean blood pressure, arterial oxygen saturation and electrocardiography using lead II were recorded every thirty minutes for the first two hours, then every one hour for 24 hours. Recording the first time of request of analgesic and total amount consumed in the 1st 24 hours in both groups was also carried out. The occurance of post operative complications was also recorded.

Statistical Analysis

The data was statistically analyzed using SPSS (version 11.5). Analysis of variance (ANOVA) was done to compare between more than two groups and student "t" test was used to compare between two groups. Paired "t" test was also done to study the changes in the same parameter in different time. For P-selectin, Wilcoxon test and Man Whitney were used for such comparisons as its values was abnormaly distributed (non-parametric analysis).

Results

Preoperative resting ECG showed no signs of myocardial ischemia. The other clinical data was shown in table 1.

The prelimanery laboratory investigations were presented in table 2, where no significant difference was found between the two groups.

Table 3 showed the pre-and post-operative levels of total CK, CK-MB and their ratio (CK-MB/ total CK), where no significant difference was found in either total or CK-MB between the two groups. The postoperative level of total CK showed significant increase than the corresponding preoperative level in both groups. No significant difference was found between the post-and pre-operative levels of CK-MB. The ratio of CK-MB/ total CK showed significantly decreased values postoperatively than preoperatively in both groups.

Table 1. Clinical Date of the Two Groups.

Items (mean ± S.D.)	Group I (n = 20)	Group II (n = 20)	*P* value
Age (years)	56.4 ± 4.24	56.35 ± 4.03	NS
Duration of diabetes (years)	11.3 ± 4.03	12.95 ± 3.93	NS
Weight (Kg)	90.3 ± 9.86	87.9 ± 6.93	NS
MABP (mmHg)	99.35 ± 6.82	93.55 ± 7.61	<0.05
Heart rate (/mm)	85.5 ± 11.33	80.7 ± 7.73	NS

MABP: Mean arterial blood pressure.

P>0.05 is not significant.

NS= Not significant

Table 2: Preoperative Laboratory Investigations in the Two Groups.

Items (mean ± S.D.)	Group I (n = 20)	Group II (n = 20)	*P* value
Hemoglobin (g/ dl)	12.2 ± 1.47	12.16 ± 1.13	NS
Hematocrit(%)	37.3 ± 3.93	37.7 ± 3.32	NS
WBC_s ($x10^3$)	7.5 ± 1.42	7.0 ± 1.11	NS
Platelets ($x10^3$)	286.1 ± 90.54	271.3 ± 37.09	NS
Prothrombin activity (%)	93.9 ± 5.62	91.7 ± 7.76	NS
Glucose (mg/dl)	154.3 ± 16.88	146.4 ± 16.79	NS
Glycated HbA_{1C} (%)	7.9 ± 1.39	7.5 ± 1.77	NS
Creatinine (mg/ dl)	1.02 ± 0.14	0.95 ± 0.14	NS
ALT (U/L)	20.5 ± 10.45	17.3 ± 9.07	NS
Total cholesterol (mg/ dl)	209.3 ± 33.67	196.9 ± 28.88	NS
LDL. C (mg/ dl)	138.1 ± 41.06	128.1 ± 16.77	NS
HDL. C(mg/ dl)	36.3 ± 12.34	34.7 ± 13.38	NS
Triglycerides (mg/ dl)	162.5 ± 105.4	166.7 ± 105.7	NS

- WBC_S: White blood cells. - ALT: Alanine aminotransferase.

- LDL-C & HDL-C: Low-density & high density-lipoprotein-cholesterol. *P*>0.05 is not significant. NS= Not significant

Table 3. Creatine kinase (CK), its MB isoenzyme and their ratio in the two groups.

Items (mean ± S.D.)	Group I (n = 20)	Group II (n = 20)	P_1
Total CK (U/L):-			
Preoperative	96.0 ± 19.9	110.0 ± 23.9	NS
Postoperative	278.7 ± 89.96	272.0 ± 106.81	NS
P_2	0.0001	0.0001	
CK-MB (U/L):-			
Pre operative	25.5 ± 6.96	22.1 ± 3.86	NS
Post operative	28.9 ± 13.74	24.7 ± 6.92	NS
P_2	NS	NS	
CK-MB/ total CK:-			
Pre operative	0.242 ± 0.08	0.235 ± 0.07	NS
Post operative	0.104 ± 0.04	0.085 ± 0.04	< 0.05*
P_2	0.0001	0.0001	

P_1: Significance between the two groups at the same time.

P_2: Significance between the same group pre- and post-operatively.

P<0.05 is significant. NS= Not significant

Table 4 showed the pre-, intra-and post-operative levels of plasma sP-selectin, where no significant difference was found in the preoperative levels in the two groups. Its levels at 15 minutes intraoperatively and one hour postoperatively showed significant increase in both groups than the corresponding preoperative levels, but the increase was more marked in group I.

Table 4:Plasma s P-selectin levels in the two groups

Level (ng/ ml)	Group I (n = 20)	Group II (n = 20)	P_1
Preoperative (baseline):-			
Min. –max.	18 – 150	25 – 144	NS
Median	37.5	37.5	
15 min. intra operative:-			
Min.-max.	49 – 385	39 – 168	< 0.05
Median	96.0	73.5	
Ih postoperative:-			
Min.-max.	63 – 298	65 – 204	0.01
Median	133.0	82.0	
P_2	0.00	0.000	
P_3	0.00	0.000	
P_4	0.001	0.003	

P_1 (Mann Whitney): significance between group I and groupII

P_2 (Wilcoxon): significance between level at 15 min intraoperatively and preoperative level in each group.

P_3 (Wilcoxon): significance between level at one hour postoperatively and pre operative level in each group

P_4 (Wilcoxon): significance between 1h postoperative and intraoperative levels in each group.

P<0.05 is significant. NS= Not significant

Discussion

The reported incidence of perioperative myocardial ischemia in patients with or at risk of coronary artery disease varies considerably, ranging from 20 to 63% (Landesberg *et al.,* 2002). Perioperative myocardial ischemia in risky patients, e.g. diabetics, tends to develop on the day of or the day after surgery (Rajagopalen *et al.,* 2007). Most ischemic episodes starting at the end of surgery and during emergency from anaesthesia (Landesberg *et al.,* 2001). The vast majority of these episodes are silent (Landesberg *et al.,* 1993).

Type 2 diabetes has long been recognized as a major risk factor for coronary artery disease and premature atherosclerosis (Wingard & Barrett-Connor, 1995; Hidle *et al.,* 2010). Myocardial ischemia in diabetic patients is often

asymptomatic and when it becomes clinically manifest, it is an advanced stage with high mortality rate (Caps *et al.,* 2000).

In the present study, the diabetic females showed no preoperative resting ECG findings. However, they still at high risk of myocardial ischemia, since it was reported that the baseline ECG is of limited value because it can be normal in up to 50% of patients with significant coronary artery disease (Mangano & Goldman, 1995).

Uncontrolled diabetes is another important risk factor for the development of myocardial ischemia. In the present study, the diabetic females in both groups showed higher mean values of glycated hemoglobin A_{1c} (7.9 & 7.5%; respectively) and 12 females in group I (60%) and 11 in group II (55%) showed levels more than 7%. This indicated a poorly controlled diabetic state and hence the liability of cardiovascular events. Gerstein (2004) reported that glycosyleted hemoglobin is an independent risk factor for cardiovascular events. Khow *et al.* (2004) found that for every 1% increase in hemoglobin A_{1C} above 7%, there is a 40% increase in the risk for coronary heart disease.

Dyslipidemia is another risk factor for cardiovascular diseases (Syvanne & Taskinen, 1997). In the present study, the mean plasma LDL-cholesterol and triglyceride levels showed higher values in both groups than that of the reference values and the plasma HDL-cholesterol showed a lower values than that of the reference value (Burtis *et al.,* 2008). These findings add more to the liability of these females for the occurrence of cardiovascular complication on surgical stress.

In the present study, the significant increase that was found in the postoperative total plasma CK activity in both groups than the corresponding preoperative levels is due to the effect of surgical trauma, since CK enzyme is found in large quantity in cardiac and skeletal muscles and that found in plasma is mainly due to muscle source and its increased level is non specific for cardiac ischemia (Burtis *et al.,* 2008; Bakker *et al.,* 1993). This was proved in the present study by the absence of significant difference in the postoperative plasma CK-MB activities in both groups than those of preoperative ones. The significant reduction in the postoperative ratio of CK-MB/ total CK, found in the present study, than that of preoperative value in both groups was due to the incease in the total CK activity.

The currently available diagnostic tools for myocardial ischemia, e. g. electrocardiogram and myocardial biochemical markers (CK&CK-MB) have several short comings, including delayed sensitivity and low specificity for the timely detection of myocardial necrosis. Therefore, the search for better method for rapidly identifying patients with unstable coronary syndrome is one of the utmost priorities of modern emergency medicine. That is why this work studied the plasma sP-selectin level as it is an early predictor of cardiovascular events (Ay *et al.,* 2008; Ikeda *et al.,* 1995; Ridker *et al.,* 2001).

It was suggested that the expression of P-selectin after injury is biphasic with an acute and modest peak appearing within 20 minutes and a significant peak by one to four hours. The initial expression of P-selectin is attributed to the mobilization of intracellular storage granules, followed by rapid internalization and degradation. The second phase is attributed to the de novo synthesis of P-selectin. The first phase of P-selectin expression makes it one of the early mediators of leukocyte-endothelial interaction. In addition, the presence of P-selectin on platelet surfaces serves to promote micro-thrombus formation in capillaries and venules of the injured tissue (Ikeda *et al.,* 1995).

In the present study, the mean plasma sP-selectin preoperative levels showed no significant difference between the two groups. Its level 15 minutes from the start of skin incision

showed significant increase than that of the preoperative level, denoting platelet activation and first phase of P-selectin expression. Its level one hour postoperatively showed more significant increase, in also both groups, than that of either pre-or intra-operative levels. This denotes the de novo synthesis of P-selectin and that these females are at high risk for the occurrence of myocardial ischemia later on. However, non of them showed postoperative evidence of chest pain or ECG changes suggestive of ischemia. This is because it is well known that myocardial ischemia in diabetic patients is often asymptomatic (Landesberg *et al.,* 1993; Caps *et al.,* 2000).

The enhanced coagulation that occur in surgical stress is due to an increased factor VIII, von Willebrand factor and fibrinogen, an inhibition of fibrinolysis and activation of platelets (Parolari *et al.,* 2005). It is well established that activated platelets binds to neutrophils and monocytes via interaction between P-selectin on platelet surface and P-selectin ligand on leukocyte surface, which is the final cellular mechanism leading to thrombus formation (Palabrica *et al.,* 1992; Freedman & Loscalzo, 2002; Hue & Xia, 2009; Jennings, 2009).

Many studies reported increased P-selectin level either peri-or post-operatively (Parolari *et al.,* 2005; Dymicka-Piekarka *et al.,* 2006; Lin *et al.,* 2009; Mu Leudzi *et al.,* 2009; Ray *et al.,* 2010). The occurrence of cardiac events (Rajagopalen *et al.,* 2007; Ray *et al.,* 2010) or venous thrombosis (Ay *et al.,* 2008; Wang *et al.,* 2010) was also reported intra-or post-operatively, especially in diabetic patients (Mu Leudzi *et al.,* 2009).

The effect of volatile anaesthesia on platelet functions and P-selectin level has been studied in several in vitro studies. One study revealed that isoflurane has no effect on platelet aggregation (Mozuchi *et al.,* 2000), while other studies showed increased expression of P-selectin on platelet surface (Frohlich, 1998; Rossi *et al.,* 2002).

On the other hand, it was suggested that administration of local anaesthesia may be able to reverse, or at least limit, perioperative hypercoagulability by preventing the release of procoagulant mediators, by inhibiting their signaling pathways, or through increased fibrinolysis (Donadoni *et al.,* 1989; Rosenfeld *et al.,* 1993). This was proved in the present study since the intra-and post-operative increase in plasma sP-selectin level in group II (who received local anaesthesia) were significantly less than their corresponding levels in group I (who received general anaesthesia). The plasma sP-selectin level at 15 minutes intra-operatively in group II showed significantly lower value compared to that in group I and its level one hour postoperatively showed also significantly lower value compared to that in group I.

The commonly used local anaesthetics suppress platelet aggregation, at least partially, as they suppressed thromboxane A_2-induced Ca^{2+} release from platelet dense tubular system without affecting thromboxane A_2 receptor function (Hirakata *et al.,* 2001).

It was reported that epidural anaesthesia showed superiority in decreasing cardiac mortality after surgery (Blomberg *et al.,* 1990; Spencer *et al.,* 1995). In addition, spinal anaesthesia with a microcatheter may be used as a primary method in high risk patients for whom general anaesthesia would be associated with higher morbidity and mortality (Kumar *et al.,* 2008).

In addition, in the present study, heart rate and mean blood pressure were significantly lower in the patients receiving paravertebral block (Group II) compared to those receiving general anaesthesia (group I), denoting the ability to abolish the stress response associated with anaesthesia and surgery. Regarding the postoperative complications, there was a significant reduction of

postoperative nausia and vomiting in group II compared to group I. As regard the first dose of postoperative analgesia required, group II showed better pain control and significantly less amount of opioid consumption in the first 24 hours, compared to group I.

In conclusion, paravertebral block as a local anaesthesia is an easy technique with few side effects and complications. Its use in high risk patients as diabetics is preferable, as an alternative to general anaesthesia, to decrease the risk of thrombotic complications.

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