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Changes in coagulation profile after low dose aspirin therapy in high risk pregnancy

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ABSTRACT

Aspirin when administered in low dose is beneficial for the prevention and treatment of pregnancy induced hypertension. But aspirin is associated with many coagulation disorders. This study has been carried out on the high risk patients who were given low dose aspirin and the coagulation factors and platelets were studied before and after treatment of aspirin. The study revealed no change in platelet count and morphology of platelets. The bleeding time was significantly prolonged but was within normal limit due to defective haemostatic plug formation. No significant change in PT, KCCT, CT or clot retraction was found. Platelet aggregation revealed: a1 (angle of primary wave of aggregation) with adrenaline (1.2 μM/L) was not changed after giving aspirin as this was due to the agonist added. A2 (angle of secondary wave of aggregation) with 1.2 μM/L adrenaline fell significantly from 32.5° to 21.66° after aspirin therapy due to failure of release reaction from platelets. A (angle of monophasic wave of aggregation) with ADP 4 μM/L also decreased from 50.4° to 30.4°. Due to high concentration of ADP only single wave of aggregation was obtained. P_{max} (percentage of maximum aggregation) decreased significantly with both agonists. With adrenaline from 42.77% to 22.3% and from 39.0% to 18.35% with ADP. No. change was seen with T_{max} (time taken for maximum aggregation).

Key words: Coagulation, Platelet, Dysfunction, Pregnancy induced hypertension, Aspirin, Low dose, Profile.

INTRODUCTION

Aspirin has been used by many for the purpose of prophylaxis in pregnancy but aspirin causes a variety of coagulation disorders even if given in low dose. Acetyl Salicylic acid (aspirin) readily crosses placental barrier so both maternal and foetal haemostasis may be affected. Aspirin even in low dose prolongs bleeding time[1] and inhibit platelet adhesion, secondary work of aggregation etc. Aspirin has been found to decrease pseudopod formation of dendritic form and formulation of spherules[2]. Aspirin causes changes in membranes, endomembrane, endoplasmic reticulum and granules; and these changes were dependent on concentration of aspirin. All doses of acetyl salicyclic acid significantly inhibit platelet function and increase bleeding times relative to control values. Maximum increase in dysfunction were obtained with daily doses of 100 mg. Minimum daily dose of aspirin was not identified, certain investigators have indicated as little as 1 mg/day and controversy still exists on the optimal dose.

Moreover, low-dose aspirin therapy (60 to 100 mg per day) reportedly reduces the incidence of preeclampsia among selected women deemed to be at risk for this

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complication on the basis of the obstetrical history[3,4], an increased sensitivity to the pressor action of angiotensin II[5], a positive rollover test[6], or abnormal findings on Doppler ultrasonography of the uterine arteries[7–9]. This protective effect of aspirin is assumed to be mediated by a decrease in thromboxane production without a reduction in prostacyclin production[10], which thus prevents the vasoconstriction and coagulation problems that are characteristic of preeclampsia[11]. Whether aspirin will benefit all pregnant women is however, unknown.

Other benefits that have been ascribed to aspirin include a lower incidence of intrauterine growth retardation and of preterm delivery and higher birth weight – all due in part to the prevention of early preeclampsia[12,13]. In still other reports, women treated with aspirin in whom preeclampsia did not develop has longer pregnancies, and their infants had higher birth weights[3,14,15].

In contrast aspirin has been implicated in a number of adverse actions affecting the mother, fetus, or neonate[12,13]. The maternal risks include increased antepartum and postpartum hemorrhage; the fetal risks include oligohydramnios; and the neonatal risks are persistent pulmonary hypertension and a variety of bleeding problems[12]. However, no increase in the frequency of adverse effects among the women in the aspirin group was reported in previous studies[3–7].

Many studies have been done on changes in coagulation profile in mother and neonates with aspirin ingestion during pregnancy. Reporting platelet dysfunction and increased bleeding tendency in neonates[16]. Stuart et al. (1982)[17] studied 4 groups of maternal-neonatal pairs for platelet count, aggregation, prothrombin time, partial thromboplastin time, thrombin time in maternal and cord blood. They concluded that if the intake of aspirin is more than 10 days before delivery there are little effects on coagulation (Sibai et al[18]). Studies were conducted on platelet aggregation in pregnancy with 60 mg/day and 80 mg/day aspirin after 1 week of therapy. Maternal platelet aggregation was inhibited with both ADP and collagen with 60 mg and 80 mg dose but not with 20 mg either 1 or 2 weeks later. 60 mg per day aspirin reduced maternal platelet thromboxane production in response to ADP (50%) and collagen (60%) after 1 week of treatment, a non-significant reduction but after 2 weeks of treatment significant decline was observed. With 80 mg dose, 99% and 98% respectively, fall was seen within 1 week. 20 mg dose did not show any fall even after 2 weeks. This study was conducted to investigate the various parameters of coagulation in patients receiving low-dose aspirin.

MATERIAL AND METHOD

A total of 421 patients were screened for high risk pregnancy induced hypertension out of which 396 could be followed till delivery. Patients were divided into low risk group and high risk group. High risk group patients were randomly divided into two groups and one group was given 50 mg aspirin and another group was given placebo. For studying coagulation parameters, 40 healthy low risk females were taken as control. The various parameters studied were absolute platelet count, bleeding time, clotting time, prothrombin time, KCCT and PF3 availability and changes in platelet aggregation. All the parameters were done before starting 50 mg of aspirin and after 4 weeks of starting aspirin. For studying the function of platelet aggregation, 40 healthy controls were also analyzed.

RESULTS

Of 396 females who could be followed till delivery, 320 (80.8%) had low risk profile while 76 (19.2%) had high risk parameters. For studying the coagulation parameters, 40 healthy low risk patients were taken as control. The various changes in absolute platelet count, bleeding time, clothing time, prothrombin time, KCCT and PF3 availability is shown in Table 1. There was no change in the platelet morphology and absolute platelet count. Bleeding time was significantly prolonged by the use of aspirin but the increase in bleeding time was still within the normal range. There was poor clot retraction in 5% controls and 8.3% cases after aspirin which was not a significant change. The clotting time, prothrombin time, KCCT and PF_3 availability did not change after aspirin therapy. Table 2 shows the changes in platelet aggregation with aspirin. There was no significant fall in a_1 with adrenaline but a_2 value fell significantly after aspirin therapy. At the concentration of 4 μM/L of ADP only monophasic wave of aggregation was obtained in all cases. There was a highly significant fall in angle of monophasic wave after giving aspirin. It was also significant with respect to control value. The P_{max} using adrenaline fell significantly after giving aspirin with respect to preaspirin value and control. Similarly P_{max} with ADP also fell significantly after aspirin. No significant change was seen with aspirin in T_{max} using adrenaline as well as ADP.

TABLE 1
Various changes after aspirin therapy

	Non aspirin (40)	Preaspirin (36)	Post aspirin (36)
Absolute platelet count ($X10^3$/cumm)	289.075±109.94	306.30±91.84	308.19±93.53
Bleeding time* (in Secs)	1.416±0.433	1.45±0.51	2.27±0.89
Clotting time (in Secs)	4.28±0.271	4.41±0.57	4.53±0.86
Prothrombin time (in Secs)	14.85±1.3444	14.75±1.73	14.86±1.73
KCCT (in Secs)	52.36±7.16	52.10±5.8	51.84±5.74
PF_3 availability	0.276±2.47	0.511±2.5	0.57±2.22

*Only significant difference in bleeding time.

TABLE 2
Changes in platelet aggregation with aspirin

	(A) Non aspirin (40)	(B) Control (40)	(C) Preaspirin (36)	(D) Postaspirin (36)
a_1 (angle of primary wave of aggregation in degree) Agonist – Adrenoline (Conc. 1.2 μM/L)	46.35±13.53	49.35±11.10	47.762±9.089	46.38±8.87
a_2 (angle of secondary wave of aggregation in degree) Agonist – Adrenaline (Conc. 1.2 μM/L)	36.8±8.10	32.3±6.2	32.5±9/0	21.66±4.7*
a (angle of monophasic wave of aggregation in degree) Agonist – ADP (Conc. 4 μM/L)	50.5±14.0	46.5±13.12	50.4±9.2	30.4±10.4*
P_{max} % of maximum aggregation Agonist – Adrenaline (Conc. 1.2 μM/L)	41.6±7.9	41.5±6.34	42.77±11.2	22.3±7.79*
P_{max} % of maximum aggregation Agonist – ADP (Conc. 4 μM/L)	42.7±8.6	39.7±8.99	39.0±10.92	18.35±4.9
T_{max} Time of maximum aggregation in seconds Agonist – Adrenaline (Conc. 1.2 μM/L)	131.5±41.9	115.5±24.09	120.8±25.8	123.11±18.01
T_{max} Time of maximum aggregation in seconds Agonist – ADP (Conc. 4 μM/L)	122.65±33.47	116.0±23.26	112.7±20.64	118.11±30.142

* p value < 0.001 between group B vs D and C vs D. Other values not significant.

DISCUSSION

Various parameters of platelet aggregation, platelet factor-3 availability, bleeding time, platelet count and morphology and clot retraction were studied. Samples were taken before starting aspirin and 3–4 weeks after initiation of therapy. No change was found in absolute platelet count after giving aspirin in comparison to pre-aspirin values and controls and also no change was seen in platelet morphology, similar to the results by Gast (1964)[19]. Clot retraction was not significantly altered after giving aspirin, poor clot retraction was found in 5% controls and 8.5% cases after giving aspirin similar to Gast (1964)[19].

Many morphological changes in membranes, granules, and endoplasmic reticulum were observed. Use of

aspirin have been reported[2] which were dose dependent. We cound not find such changes which may be because of low dose of aspirin (50 mg/day) and less resolution with our microscopes. Clot retraction is a function directly proportional to platelet count and inversely proportional to fibrinogen concentration. As low doses of aspirin affect both values, no effect was noted in this study. Bleeding time was significantly prolonged after therapy in our study. Gast, (1964) also used low dose aspirin 30–60 mg/day and their results were comparable to our data[19]. This may increase bleeding tendency of the patient during labour or in puerperium. Very prolonged bleeding time with 50 mg/day dose has been reported[1].

Platelet aggregation was studied using ADP and adrenaline as agonist. With ADP only a monophasic wave could be obtained. This was because of high concentration of ADP used. Atac et al. (1970) also reported that ≥ 10 μM ADP concentration, primary and secondary aggregation can not be differentiated and only at a critical concentration of 1 μM both waves were observed, though it varies with different PRP samples[20]. With adrenaline biphasic curves were obtained. A significant fall in secondary wave of aggregation was seen after aspirin both with ADP and adrenaline[21]. Angle of secondary wave of aggregation [(a_2) and a in case of ADP] fell significantly. The effect was more apparent with adrenaline than ADP[7]. Primary wave of aggregation was not affected with either agonist as a_1 remained unaltered[21].

The percentage of maximum aggregation P_{max} was also reduced significantly from 42.7% to 22.3% with adrenaline and 39% to 18.3% with ADP. Sibai et al[18], showed a decrease of 64% from 73% after 2 week treatment with 60 mg aspirin. No change was seen in time taken for maximum aggregation with either agonist. It has been shown that primary wave of aggregation is caused by the agent added while the second wave is caused by release of intrinsic platelet ADP. The platelet aggregation response to ADP and epinephrine after aspirin intake is limited to single wave of 'reversible' aggregation and both 'oxygen burst' and release of serotonin, ADP, ATP, PF-4 etc. is abolished. Aspirin acetylates aggregates proteins and stabilizes lysosomes and has general inhibitory effect on membrane permeability. All these actions will lead to inhibition of secondary wave of aggregation which can prove useful in conditions marked with hypercoaggulability.

Platelet factor-3 availability was not changed significantly in our study after giving aspirin. This could be because of low dose of drug. Al-Mondhiry et al. (1969)[22] found that platelet factor-3 activation by critical concentration of ADP was abolished by 20 μM concentration of aspirin in vitro. Decrease in PF-3 availability was also found[23]. Atrac et al. (1070)[20] studied PF-3 activity with various concentrations of ADP with use of 60–325 mg of aspirin ingested for ≥ 2 weeks. They found decrease only with optimal concentration of ADP. With 1–10 μM no change was seen. The finding suggests that development of PF-3 activity depends upon the degree of aggregation, whether primary or secondary.

No change was found in PT, KCCT and clotting time. Prothrombin time was not significantly prolonged with low dose of aspirin[19]. Platelet angiotensin II receptor status has been studied but it failed to provide the significant information regarding the risk of PIH[24]. It appears that parameters like PT, KCCT and clotting time are dependent on many coagulation pathways they remain unaffected by low doses of aspirin. A clinically significant change was not found in coagulation profile.

Low doses of aspirin selectively inhibit synthesis of platelet thromboxane causing improvement in TXA_2/PGI_2 ratio which is the pivotal cause in pathogenesis of PIH. This study shows that even at low doses, 50 mg/day aspirin prevents secondary wave of aggregation of platelets by inhibiting release of ADP using both agonist i.e. adrenaline and ADP. Both the angle of secondary wave (a_2) and percentage of maximum aggregation P_{max} were significantly reduced. T_{max} remained unchanged. Bleeding time was significantly prolonged though other parameters of haemostasis remained unchanged.

The efficacy of aspirin in the prevention of arterial thrombosis has been known for many years[25,26]. Several studies have suggested that low-dose aspirin can prevent pre-eclampsia and/or fetal growth retardation. There were significant reductions in the frequencies of pre-eclampsia, fetal growth retardation, and fetal death[3]. Other controlled[5,10,15,25] and randomized[14,26] studies supported such an effect of aspirin.

The effect of aspirin is linked to an action on prostaglandins. Low-dose aspirin greatly inhibits thromboxane production in human placental arteries in vitro, but has no effect on production of prostacyclin[27,28]. In vivo[10,29], low-dose aspirin selectively reduces urinary excretion of thromboxane B_2, without changing that of 6-keto-prostaglandin $F_{1\alpha}$. Some studies[10,29] have found reductions in fetal thromboxane with aspirin, and others[11] have not. Sanchez-Ramos et al[30] reported a significant decrease in angiotensin II pressor responsiveness 2 h after ingestion of 80 mg acetylsalicylic acid by pregnant women in the third trimester. Spitz et al[31] showed that the same treatment greatly increased the pressor dose of angiotensin II,

mirroring a reduction of thromboxane B_2, in pregnant patients with a positive angiotensin test.

Despite its potential benefits, aspirin use has been discouraged in pregnant women because of a possible risk of abnormal bleeding in the mother or the newborn infant, and because of some doubts about congenital defects in babies. Aspirin does not increase the overall risk of malformations, and cardiac defects are not associated with aspirin use[32]. The risk of bleeding, especially in the infant, has been emphasised when mothers ingested high doses of acetylsalicylic acid[33,34]. No study of low-dose aspirin has reported abnormal bleeding in babies. Moreover, Sibai et al[11] showed good clinical and biological tolerance in babies whose mothers ingested 60–80 mg aspirin. Those infants also had patent ductus arteriosus and a normal right ventricular systolic time interval ratio. Thus, there is as yet no evidence of a definite risk associated with low-dose aspirin in pregnant women. However, data are still scarce and great caution remains necessary.

In conclusion, this study on the assessment of haemostatic markers in high-risk pregnancies could provide evidence on the efficacy of early low-dose aspirin in preventing fetal growth retardation and maternal proteinuria. Its effects on fetal death and abruptio placentae have not been proved as yet, and would need to be tested by specific trials. Thus, it now seems justifiable to propose aspirin treatment for any patient considered at high risk, even if in her first pregnancy. On the other hand, massive use of aspirin by millions of pregnant women yearly certainly cannot be recommended. Early, reliable, and inexpensive markers of risk need to be identified urgently. Although preliminary data are reassuring, only the very large scale clinical trials going on in several countries can prove the safety of aspirin. Meanwhile, indication and the benefit versus risk ratio should be weighed with great caution for each patient.

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