The Impact of Low Dose Aspirin on Markers of Inflammation and Placental Function: An Ancillary Study of the ASPIRIN Trial

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Abstract

Objective: To determine the impact of low dose aspirin (81mg) on markers of maternal inflammation and placental function. Setting: Rural Southern India Population: Nulliparous women with a singleton pregnancy dated by ultrasound who were enrolled in the ASPIRIN (Aspirin Supplementation for Pregnancy Indicated risk Reduction In Nulliparas) Trial. Methods: We performed a case control study of women who delivered prematurely compared to term controls in women enrolled in the ASPIRIN trial. Women were prospectively enrolled in an ancillary observational trial wherein maternal serum was collected and measured between 10 to 13 weeks and 17 to 21 weeks of gestation after initiation of aspirin or an identical placebo. Our primary outcome was the impact of aspirin on markers of placental function and maternal inflammation. Results: From 2016-18 with a total of 666 n women enrolled in this ancillary trial of whom 269 were selected for analyte analysis. Women who received low dose aspirin (LDA) had lower levels of Alpha Feto-Protein (AFP) at 10 to 13 weeks than women who received placebo (Placebo) (LDA 18.3 ng/mL vs 21.4 ng/mL -P 0.001). AFP was similar between the two groups at 17 to 21 weeks. No other differences were seen in in C-Reactive protein or Anti-Mullerian Hormone. Conclusion: Low dose aspirin administration lowers AFP early in pregnancy and may be a marker of Aspirin efficacy. Keywords: maternal serum alpha-fetoprotein (MSAFP), preterm birth, aspirin Tweetable Abstract: Aspirin decreases AFP in the first trimester; a marker associated with poor birth outcomes.

INTRODUCTION:

Background: The World Health Organization estimates that 15 million children are born prematurely each year resulting in the second leading cause neonatal mortality throughout the world and in particular low-middle income countries[1,2]. In addition to its significant contribution to mortality, the impact of preterm birth amongst children born prematurely often continues throughout their lifespan. Compared to children born at term, children born prematurely have higher rates of cerebral palsy, milestone delay, impairment in learning, visual disorders and higher rates of long-term physical health problems[3–5].

Recently, the Aspirin Supplementation for Pregnancy Indicated Risk Reduction In Nulliparas (ASPIRIN) trial demonstrated that nulliparous women with no more than two previous first trimester pregnancy losses treated with Low Dose Aspirin(LDA) 81mg daily beginning between 6 0/7 weeks and 13 6/7 weeks through 36 0/7 weeks gestational age have lower rates of preterm birth from all causes and a decrease in perinatal mortality[6] .Though the ASPIRIN trial holds promise of a therapeutic option for the prevention of preterm birth, the mechanism by which it works has not been fully elucidated. LDA has been well chronicled to

affect both the COX-1 (thrombosis pathway) and the COX-2 (inflammatory pathway); however, the exact pathway through which it exerts its affect remains uncler[7,8]. We thus sought to examine the impact of LDA on both inflammatory markers and established markers of placental function amongst women who received either LDA or placebo in the ASPIRIN trial.

MATERIAL AND METHODS

We performed a case control study of women enrolled within the study within the ASPIRIN Trial conducted at the J N Medical College, Belagavi Karnataka India. (JNMC Women's and Children's Health Research Unit; Global Network for Women's and Children's Health Research). Specifically, we identified women who delivered before 37 weeks (Cases) and women who delivered at term (controls). As this analysis was exploratory in nature with no prior data documenting differences due to aspirin treatment in pregnancy, a power analysis was unable to be calculate. We thus established a goal of including approximately 50 preterm birth cases and 150 term controls. Either sample limitations or technical challenges in laboratory measurement, intermittently precluded achieving the full 50/150 ratio. Due to sample limits, a separate either preterm or control case was chosen rather than having a fixed cohort of patients used for all specimens. The study protocol was approved by the ethics committee of the institution and women were individually consented for participation. As part of the primary study, women were included if they were nulliparous with no more than 2 prior pregnancy losses, pregnant with a singleton pregnancy between 6 weeks and 13 weeks and 6 days as verified by ultrasound. Women with foetal anomalies or medical conditions contraindicated to receive LDA were excluded. Following randomization to either LDA or placebo, serum was obtained and stored at -80 ^{0}C degree until analysis. A second sample was obtained at 17-21 weeks gestation and similarly stored. Women who experienced an abortion prior to 16 weeks were excluded.

Measurements of the various analytes were performed using standardized immunometric immunoassay (Alphafetoprotein [AFP]- R&D Systems, kit# DAFP00; Anti-Mullerian Hormone [AMH]-Catalog Number: DY1737 R&D Systems) and Immunoturbidimetric (C-Reactive Protein [CRP]- Roche Diagnostics) methods in accordance with the kit manufacturers protocol.

Statistical analysis:

Our primary outcome was preterm birth defined as delivery before 37 weeks. Secondary outcomes of interest were PTB <34 weeks and hypertensive disorders of pregnancy. All continuous variables were assessed for normality using the Shapiro Wilk test. Bivariate analysis was performed using the Wilcoxon Rank Sum test where appropriate. Additionally, we compared the impact of LDA on these biomarkers on these analytes using a Wilcoxon Rank-Sum Test. All analyses were complete using STATA v15.1 (Colleges Station, TX).

RESULTS:

A total of 666 women participated in this investigation of which a total of 7 experienced an abortion prior to 16 weeks. A total of 269 women had sample results that were able to be included (45 preterm and 224 term). Demographic data of the total cohort are displayed in table 1. Maternal demographics were noted to be similar between the two groups. Consistent with the results of the parent trial, women who received Aspirin had lower rates of preterm birth but this did not meet statistical significance in this much smaller cohort enriched with preterm birth cases. Similarly, birthweight tended to be greater in the Aspirin group and conversely the rate of small for gestational age (SGA) and birthweight <2500gm were lower in the Aspirin group. Of note the rate of birthweight <1500gm was noted to be similar.

Results of the analytes are displayed in table 2. Notably none of the values were normally distributed by the Sharipo-Wilk test and therefore only Wilcoxon Rank-Sum tests are reported. No difference was seen in the values of any of the analytes as they related to either preterm birth or hypertensive disorders of pregnancy; excepting that AFP was found to be lower amongst mothers who delivered before 34 weeks at the time of the first blood draw. LDA use was also associated with markedly lower levels of AFP at the first time point that blood was drawn. Neither of these differences persisted at the second time point.

The relationship of the varied measured analytes on gestational age is displayed in table 3. Only C reactive

protein at the first visit was shown to correlate with gestational age. This result was noted to be positive suggesting that higher-Reactive Protein is associated with longer gestational ages.

DISCUSSION

The results of our investigation suggest several interesting tenets about how the role in Aspirin in pregnancy. First and foremost, we noted that AFP is decreased at the time of the first blood draw amongst women who took aspirin compared to those who did not take aspirin. This difference was not noted at the second blood draw. AFP has been well documented to be associated with poor obstetrical outcomes including both preterm birth and ischemic placental diseases[9,1011–14]. Though our limited data did not show a correlation of first trimester AFP with any obstetric outcomes, this is probably a reflection of sample size. This data is suggestive that aspirin plays an important role in facilitating deep placentation[15,16] and that it's early initiation is important as this process occurs largely before 18 weeks. Several meta-analyses have suggested that early initiation of aspirin is key to avoidance of preeclampsia[17,1819,20]. This would suggest that measurement of AFP in the first trimester may be a useful biomarker to understand the efficacy of LDA in pregnancy; however, larger validation studies are necessary.

In contrast to AFP, we saw no differences in the levels of either AMH or CRP at either gestational time points. It should be noted that AMH was lower at both time points among women taking aspirin but neither was statistically significant. AMH has been shown by others to be associated with preterm birth; albeit it has mostly been evaluated in the setting of women with infertility issues which may intersect with other medical conditions[21,22]. Finally, C-reactive protein is an acute marker of inflammation and may not be predictive of obstetrical conditions.

Our study has several strengths and weaknesses. First this is an ancillary study on a well described prospectively phenotyped group of women and masking to treatment allocation was maintained throughout the study. Secondly, gestational age was validated using early ultrasound. Weaknesses of our study include the fact that overall numbers of specimens was variable based upon technical challenges with the assays. Additionally, our overall sample size is limited and therefore we may lack adequate power to detect meaningful differences.

In conclusion, our study suggests that AFP in the first trimester is reduced by LDA therapy. Unfortunately, most likely due to sample size, we are unable to demonstrate the predictive effects of AMH, AFP and CRP on important obstetrical outcomes.

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The authors report no conflict of interest

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Table 1: Maternal Demographics & Birth Outcomes

	Aspirin (N= 129)	Placebo(N=146)	P-value
Demographics	Demographics	Demographics	Demographics
Age	20.6+/-3.0	21.0 +/- 3.2	0.38
Education			
None	0.8%	5.5%	
Primary	3.1%	4.1%	
Secondary	81.4%	77.4%	
University+	14.7%	13.0%	0.16*
Height Cm	151.8 +/- 5.5	151.7 +/- 5.4	0.90
Weight Kg	47.1 +/- 8.2	47.1 +/- 7.5	1.0
BMI	20.4 +/- 3.4	20.4 +/- 3.0	0.98
Prior Abortion	0.11 +/- 0.34	0.10 +/- 0.32	0.78
Hb on enrollment	11.6 +/- 1.4	11.4 +/- 1.4	0.28
Gestational Age at sample 1	11.6 +/-2.6	11.7 +/-5.1	0.80
Gestational Age at sample 2	18.7 +/-2.2	19.6 +/-3.8	0.16
Birth Outcomes	Birth Outcomes	Birth Outcomes	Birth Outcomes
Gestational Age Weeks	37.7 +/- 5.2	37.6 +/- 5.4	0.90
Birth Weight (gm)	2759	2588	0.005
PTB <37 weeks	12.1%	18.4%	0.18
PTB <34 weeks	7.8%	7.5%	0.94
Stillbirth	3.2%	5.7%	0.39*
Hypertensive Disorders of Pregnancy (HDP)	11.3%	10.6%	0.87
Small for Gestational Age	27.9%	45.0%	0.006
<2500 gm	15.7%	34.8%	0.001
<1500 gm	1.7%	4.4%	0.29*

*Fisher's Exact Test

Table 2: Serum Analytes

	Preterm	N
AFP1	18.3 +/- 9.9	41
AFP2	64.9 +/- 62.5	29
CRP1	1.9 +/- 1.8	39

CRP2	3.3 +/- 3.3	25
AMH1	2.0 +/- 1.8	40
AMH2	1.7 +/- 1.2	24
	PTB<34 Weeks	
AFP1	16.4 +/-6.9	15
AFP2	65.7 +/- 41.0	9
CRP1	1.4 +/- 0.8	13
CRP2	3.7 +/- 4.2	8
AMH1	2.2 +/- 1.6	14
AMH2	1.5 +/- 1.1	9
	HDP	
AFP1	22.3 +/-14.9	25
AFP2	67.8 +/-47.3	16
CRP1	2.1 +/-1.8	23
CRP2	3.4 +/-2.3	13
AMH1	1.7 +/-1.7	26
AMH2	1.2 +/-1.2	14
	Aspirin	
AFP1	17.3 +/- 12.3	92
AFP2	60.2 +/- 34.1	59
CRP1	2.2 +/- 1.8	82
CRP2	3.1 +/- 2.5	50
AMH1	2.1 +/- 1.4	92
AMH2	1.4 +/- 1.1	54
NB: All P-values are Wilcoxon Ranks Sums	NB: All P-values are Wilcoxon Ranks Sums	NB: All P-values are Wilcoxon

Table 3: Analytes and Gestational Age

Analyte	Coefficient (weeks/unit)	95% CI	p-value
AFP1	0.054	-0.002 to 0.11	0.06
CRP1	0.44	0.14 to 0.88	0.043
AMH1	-0.12	-0.64 to 0.40	0.65
AFP2	0.002	-0.12 to 0.16	0.73
CRP2	-0.02	-0.25 to0.21	0.87
AMH2	-0.033	-0.51 to 0.45	0.89