Evaluationofthebiologicalresponsetoacetylsalycilicacidbyplateletocclusiontimein pregnantwomeninBrazzaville

Abstract

Low-dose acetylsalicylic acid (ASA) is recommended by the WHO for pregnant women toprevent thrombotic phenomena. Despite the variability of its clinical efficacy(resistancephenomena),itsnon-standardizedbiologicalmonitoringcanbeperformedusingplateletocclusiontime(POT).Theaim ofthis study wasto assesstheresponsetoASA using POT.

Amulticenter,cross-sectional,analyticalstudywasconductedintheobstetricsandgynecology departments of six Brazzaville hospitals over a period of 09 months, and includedpregnantwomenonASA100mgdailyforatleast7days.TOPwasmeasuredusingthePFA-200. The variables studied were clinical (age, medical and obstetrical history) and biological(blood count, PTWT). Non-response to ASA was defined by a TOP of 150 seconds or less.Data analysis was performed using STATA 12 software. Logistic regression was used toassessthedeterminants associated with non-response.

The study involved 39 pregnant women, mean age 33.9 ± 5.4 years, undergoing ASA forgestationalhypertensivedisorders(48.7%)andchronichypertension(20.5%).Non-responsetoASAwasfoundin12pregnantwomen(30.7%).Nostatisticallysignificantdifferenceswereobservedbetweennon-respondersandresponderswithregardtoepidemiological,clinicaland haematological determinants (p>0.05).

Non-response to ASA, present in a third of hypertensive pregnant women, is associated withtheoccurrenceof obstetricalcomplications in Brazzaville.

Keywords:pregnancy,acetylsalicylicacid,PFA-200,biologicalresponse.

Introduction

Low-dose acetylsalicylic acid (ASA) is recommended by the World Health Organization(WHO) for the prevention of thrombotic phenomena in gestational hypertensive disorders orantiphospholipid antibody syndrome [1]. Hypertensive disorders in pregnancy are a publichealth problem, as obstetric morbidity and maternal mortality due to hypertension are high inEuropeanandAfricanstudies,andparticularlyinCongolesestudies[1,2].ASA,anantiplatelet agent, acts by irreversibly acetylating cyclooxygenase (COX-1) after a minimumofoneweek'sdailyadministration[3].However,ASA-basedthrombosispreventionhasshownsomevariability inits clinical andbiological efficacy.

Indeed, the concept of "ASA resistance" has been introduced, defined as the inability of ASAto inhibit Thromboxane A2 formation or platelet aggregation. It has been reported to affectbetween 5% and 60% of the general population [4]. Its diagnosis can be made on the basis ofmethods for assessing the biological response to ASA. Among these, measurement of plateletocclusion time (POT) simulates in vitro the hemodynamic conditions of platelet adhesion andaggregation.Furthermore,antiplateletagentsaregenerallyprescribedblindly,unlikeanticoagulants,whichbenefitfromstandardizedbiologicalmonitoringtocontroltheirefficacy and safety [5]. Thus, with the aim of contributing to the management of hypertensivedisorders in pregnancy, the objectives of this study were to assess the biological response toASAby TOPand to identifyits involvementinadverseobstetricoutcomes.

Patientsandmethods

Thiswasamulticenter,cross-sectional,analyticalstudyconductedfromMarch1toNovember 30, 2021 in the gynecology-obstetrics departments of six Brazzaville hospitals. Itinvolved all pregnant women who had been taking ASA for at least seven days, or who hadstopped taking it less than seven days previously. The ASA used was a soluble powderadministeredatadoseofone(1)100mgsachetperday.Pregnantwomenwithaplateletcount of less than 100 giga/l and/or a hematocrit of less than 10% were excluded. Theselectionof pregnantanimals was exhaustive.

Once selected, some gestational carriers were referred to the Centre National de Référence dela Drépanocytose for platelet occlusion time. Others were sampled at their follow-up centers.All patients rested for at least 10 minutes, but did not have to fast before blood sampling.Blood was drawn by venipuncture at the elbow or at the non-perfused wrist for hospitalizedpatients. We collected five (05) ml of whole blood under vacuum in tubes containing ethylenediamine tetra acetic acid (EDTA) or sodium citrate respectively.

Specimens were transportedtoroomtemperaturewithinthreehoursandusedforbloodcountandplateletocclusiontime

(POT).POTwasdeterminedusingcollagenkitscombiningepinephrine(COL/EPIINNOVANCEPFAP2Y®)andadenosinediphosphate(COL/ADPINNOVANCEPFAP2Y®)and theINNOVANCE® PFA®-200 system.

The variables studied were obstetric (gestational age, parity, spontaneous miscarriage, gravidhypertensivedisorders,prematurity,fetaldeathinutero,intrauterinegrowthretardation),therapeutic(indicationforASA,durationoftreatment)andplateletocclusiontime.Thereference values of the TOP based on the collagen/epinephrine kit ranged from 80 to 150seconds,sonon-responsetoASAwasdefinedbyaTOPlessthanorequalto150seconds,andresponseto ASAby alengthening ofthe TOP greaterthan 150 seconds.

Data were analyzed using Stata 12® (College Station, Texas 77845, USA). Pregnant women'scharacteristics were presented for continuous variables as mean and 95% confidence intervalwhenthedistributionwasnormal,andasmedianandinterquartilerangewhenitwasabnormal; for categorical variables as frequency. The logistic regression model was used toidentify the determinants of ASA resistance. Variables with a value of $p<0.20$ in univariateanalysis were retained for multivariate analysis. The results of the logistic regression modelwere presented as Odds Ratio (OR). For each of these factors, the proportional hazardshypothesis was tested using the Schönefeld residuals test. For all statistical analyses, thesignificancethreshold was set at $p<0.05$.

Results:

Thirty-nine pregnant women, with an average age of 33.9 ± 5.4 years and extremes of 22 and42 years, were treated with ASA for gestational hypertensive disorders (48.7%) and chronicarterialhypertension(20.5%).Amongthem,non-responsetoASAwasfoundin12gestational carriers (30.7%). The mean gestational age at sampling was 26 SA +2 days ± 7.9SA,andthemediantermoffirstprenatalcontactwas13SA[12;15],withextremesof7SA +2 days and 16SA +5 days. The median platelet occlusion time (POT) in non-responders andresponders to ASA was 121 seconds [101.5-141.5] and 200 seconds [168-239] respectively($p<0.05$). No statistically significant differences ($p>0.05$) were observed between ASA non-respondersandresponderswithregardtoepidemiological,clinicalandbloodcountdeterminants (Table I). The age range 30-39 years, and nonresponse to ASA were factorsassociated with the occurrence of obstetric complications (Tables II and III). The probabilityof complications in non-responders and responders was 72.2% (95% CI: 44.3-93.9%) and23.4%(95% CI:11.2-45.1) respectively, with $p=0.004$ (figure1).

Discussion

Thebiologicalresponsetosalicylicacid(SAA),studiedmainlyinvascularpathologies(peripheral vascular disease, coronary artery disease or stroke), makes it possible to assess theefficacyofSAA-basedthrombosisprevention.Thisstudy,carriedoutinthegestationalperiod, identified the phenomenon of nonresponse to ASA with PFA®-200 and assessed itsepidemiological,clinical andhematological determinants.

Among the 39 pregnant women in our study, biological non-response to ASA was found inalmost a third (30.7%) of the population studied. This phenomenon is often unrecognized inour daily practice, and under-diagnosed due to insufficient technical resources. This frequencyiswithintherangeofthose(5and60%)reportedinthegeneralpopulation[4].Itiscomparable to those found by the teams of Caron et al in Canada [6] and Jeske M. et al in theNetherlands [7], which are respectively 28.7% (assessed using PFA®-100 in 87 gestations in2007) and 30.4% (assessed using PFA®-200 in 23 gestations in 2020). However, this figure issignificantly lower than that found in the 2012 study by Leila Abid et al in Tunisia, in whichresistance to ASA was found in 54.4% of patients [8]. These results may be explained by thedifference between the populations in their studies, which consisted of patients with coronaryartery disease of both sexes. In two more recent studies conducted in the UK, the prevalencesof ASA non-response reported in the second trimester of pregnancy were 14.7% and 36%respectively. Their results differ from ours due to the methods used to measure ASA non-response, which were serum TXB2 assay for Vinogradov et al in 2021 [9] and urinary 11-dehydrothromboxane B2 assay for Navaratnam et al in 2017 in the second study [10].Indeed,theassaymethodsusedbythelatterinthedetectionofnon-responsetoASAmorespecifically identify the phase of primary hemostasis not inhibited by ASA, notably that ofplateletactivation [11,12].

Withregardtothedeterminantsofnon-response,nostatisticallysignificantdifferences(p>0.05)wereobservedbetweennon-respondersandresponderstoASAintermsofepidemiological, clinical and haematological factors (Table I). The mean age of pregnantwomen in our study was 33.9 ± 5.4 years [22y-42y]. This is similar to that reported by Caronet al in 2007 and Rey et al in 2012 in Montreal, where it was 33.6 ± 5.4 and 32.3 ± 5.0respectively. These similarities may be explained by the fact that the latter also worked onobstetric populations taking ASA for an obstetric indication like ours [13,14]. The majority ofour pregnant women were overweight or obese (48.7% and 35.9% respectively). Their medianbodymassindexwascomparabletothatfoundbyReyetalinCanada,whichwas28.3kg/m2. However, the proportion of obese subjects (49.5% versus 35.9%) was higher than ours[14]. These differences may be explained by lifestyle, and in particular by poorer eating

habitsincountries where26.8% of theadultpopulation is obese[15].

The mean gestational age at initiation of ASA treatment was higher in our series (14.3SA±4.6) than those reported by Caron et al (10.0±4.4SA) and Rey et al (9.9±3.7SA) [6,14].This is because prenatal contacts in developed countries start earlier, and in their studies,high-risk gestational carriers, once identified, could benefit directly from ASA treatment at adateimposedbythestudymethodology,whichinthiscasewasearlierthanours.Themajority of patients were recruited in the second trimester of pregnancy, with a mean age atthe time of platelet occlusion time (POT) of 26 weeks' amenorrhea and 2 days ± 7.9. This ageisconsiderablyhigherthanthatreportedinCanadianseries[13,14].Infact,themethodologies used in the latter series required that pregnant women be recruited at the firstprenatal contact (which was earlier than for our participants), and a first TOP was performedon this occasion. The Caron et al study was interventional, with a TOP performed on the dayof inclusion, 15 days later and between 24 and 32 weeks of amenorrhea, in order to studyvariationsinTOPasafunctionofgestationalage.Whenapatientunresponsiveto81mgASA was identified, the dose of ASA was increased and a sample taken 15 days later. Thesame pattern was followed by Jeske M. et al. in Amsterdam, who also performed OPT in the1st,2nd and 3rdtrimesters of pregnancyand at threemonths postpartum[7].

The TOP averages observed in the first two trimesters in our work were respectively close toandslightlyhigherthanthoseobservedinthethirdtrimester.Studieshaveshownthatpregnancy induces shortening of the OFR determined on the basis of the collagen-epinephrinekit by PFA®-100 [16] and, more specifically, from the 2nd trimester onwards in medication-free pregnant women. Caron et al found similar results in pregnant women on ASA, with ashortening of TOP from the 2nd trimester onwards [6]. Jeske et al observed a greater decreasein the 3rd trimester, with a mean of 207.87±15.4 seconds using the PFA-200 [7]. TOP valuescorresponding to the diagnosis of ASA resistance may fluctuate during pregnancy due to theexpansion of plasma volume, changes in blood cell counts, coagulation factors and steroidhormonesduring pregnancy.

This resistance to ASA may be due to several factors. A normal erythrocyte count facilitatesplatelet aggregation by stimulating prostaglandin formation by the platelet, and by recruitingneighbouringplatelets,despitetheintakeofASA.Inaddition,anormalmonocyteormacrophage count capable of expressing COX-2 provides the platelet, whose COX 1 iseffectively inhibited by ASA, with the PGG2 and PGH2 required for TBX formation. Thus,despite the effectiveness of ASA in inhibiting COX-1, inhibition of platelet aggregation isreduced. These cells induce platelet aggregation by supplying TXAS substrates, promotingdegranulation or producing TxA2 via COX-2, which is insensitive to inhibition by low

dosesofASA[2].Then,intheeventofendothelialdysfunction(atherosclerosis,

hypercholesterolemia, diabetes, smoking and arterial hypertension), NO production is reducedand its inactivation accentuated [17]. The resulting NO deficiency could translate into a stateof platelet hyperactivity, which could explain aspirin's inability to inhibit platelet activity insomeindividuals.Theseabnormalitiesresponsibleforendothelialdysfunctionmayalsocontribute totheproductionofisoprostanesathighlevels,asaresultoftheimbalancebetween oxidants and antioxidants [17, 18, 19, 20]. Among the isoprostanes formed, 8-iso-PGF2a binds to the TBX platelet receptor and activates platelet aggregation. Thus, it couldbypassaspirin'sinhibitionofTxformationandresultinsustainedplateletactivation,explaining apparentresistancetoASA.Finally,geneticvariabilitygeneratedbysinglenucleotide polymorphism can explain variation in a drug's effect [21]. This polymorphism isobserved in receptors such as GPIIb-IIIa, collagen and TXA2 receptors, and in enzymes(COX-1, COX-2, TXA2 synthetase). Differences in the gene coding for platelet COX, theADP receptor P2Y1 or GPIIb/lIIa may also explain the variability in platelet response to ASA[2].

Conclusion

Acetylsalicylicacidisoneofthemostwidelyusedprophylacticsforthromboticcomplications in pregnant women in our practice. Non-response to this prophylaxis accountsfor almost a third of pregnant women seen in obstetrical settings in Brazzaville. However,there is no national guide including a precise therapeutic algorithm with options for screeningtests, ASA dosage and complementary tests.The absence of epidemiological, clinical orhaematological determinants associated with this condition opens the door to molecular andgeneticstudies to elucidateits mechanisms.

Ethicalconsiderations

Thestudywasconductedanonymouslyandanethicalopinionwasobtained(369/MERSIT/IRSSA6 CERSA).

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Table I : Distribution of epidemiological, clinical and blood count determinants accordingtobiological responseto ASA

	Pregnantwomen		OR[IC95%]	*p*
	non-respondents n=12	respondents *n*=27		
Epidemiological				
IMC(%)				
normal	5(12.8)	9(23.1)	Reference	
overweight	4(10.3)	15(38.5)	0.48[0.1-2.2]	0.3
obesity	3(7.7)	3(7.7)	1.8[0.2-2.5]	0.5
Clinics				
Comorbidities(%)	3(7.7)	10(25.6)	0.56[0.1-2.5]	0.4
	17(43.6)	9(23.1)	Reference	
Prematurity(%)	2(5.1)	3(7.7)	1.6[0.2-11.08]	0.6
FCS*(%)	6(15.4)	9(23.1)	2 [0.5-7.5]	0.3
	6(15.4)	18(46.1)		
MFIU** (%)	1(2.6)	7(18)	0,2 [0.2-2.3]	0.2
	20(51.28)	11(28.2)	Reference	
THG*** (%)	8(20.5)	17(43.6)	1.17[0.7-8.8]	0.8
	4(10.3)	10(25.6)	Référence	
Bloodcount				
Anemia(%)				
slight	3(7.7)	12(30.8)	Référence	
moderate	6(15.4)	8(20.5)	2,9[0.5-15.6]	0.1
severe	3(18)	7(7.7)	1.7[0.2-10.9]	0.5
Hyperleukocytosis(%)	1(2.6)	2(5.1)	1.13[0.09-13.8]	0.9
	11(28.2)	25(64.1)	Référence	
Moderate thrombocytopenia(%)	3(7.7)	12(30.8)	0.41[0.9-1.8]	0.2
	9(23.1)	15(38.5)	Ref	

FCS*:spontaneous miscarriage, MFIU**: foetaldeathin utero,THG***: gravid hypertensive disorders

TableII:Univariateanalysisofclinicalandbiologicalfactorsinrelationtoobstetricalcomplications inpregnantwomenreceivingASA

Features	Pregnantwomenon ASA		HazardRatio	IC à95%	*p*
	with obstetricalcomplications n (%)	without obstetricalcomplications n (%)			
Clinics					
Ageranges					
20– 29 ans	3 (7,69)	7 (17,95)	Réf		
30– 39ans	4 (10,26)	5 (12,82)	2,32	[-]	
>40 ans	4 (10,26)	4 (10,26)	1,87	[-]	
IMC					
(kg/m2)Normal	6 (15,38)	8 (20,51)	Référence		
Overweight	7 (17,95)	12 (30,77)	0,6		
Obesity	6 (15,38)	0 (0,00)	2,43	[-]	
Gestitépri					
migest	2 (5,13)	3 (7,69)	Reference		
Paucigeste	10 (25,64)	8 (20,51)	1,51	[-]	
Multigeste	7 (17,95)	9 (23,08)	1,11	[-]	
Parity					
Nulliparous	5 (12,82)	5 (12,82)	Reference		
Primiparous	5 (12,82)	4 (10,26)	0,99	[-]	
Pauciparous	6(15,38)	10(25,64)	0,29	[-]	
Multipara	3 (7,69)	1 (2,56%)	0,88	[-]	
FCS	8(20,51)	7(17,95)	1,005	[-]	
	11(28,21)	13(33,33)	ref		
Premature delivery	3(7,69)	2(5,13)	1,21	[-]	
	16(41,03)	18(46,15)	Reference		
MFIU	3(7,69)	5(12,82)	0,77	[-]	
	16(41,03)	15(38,46)	Reference		
THG	12(30,77)	13(33,33)	1,14	[-]	
	7(17,95)	7(17,95)	Reference		
1sttrimester	15(38,46)	18(46,15)	Reference		
	4(10,26)	2(5,13)	1,21	[-]	
Biological					
Anemia					
Slight	7(17,95)	8(20,51)	Reference		
Moderate	7(17,95)	7(17,95)	0,97	[-]	
Severe	5(12,82)	5(12,82)	1,91	[-]	
Leucocytes $\geq 10^4/mm^3$	2(5,13)	1(1,56)	1,76	[-]	
	17(43,59)	19(48,72)	Reference		
Thrombocytopenia	5(12,82)	11(28,21)	0,49	[-]	
	14(35,9)	9(22,07)	Reference		
Noresponseto ASA	9 (23,08)	3 (7,69)	3,46	1,38 - 8,67	0,008
	10 (25,64)	17(43,59)	Reference		

TableIII : Multivariate analysis of clinical and biologicalfactors in relationto obstetrical complicationsinASA-selectedwomen

	Hazardratio	**IC à 95%**	***p-value***
Agerange30to 39years	19,35	0,94-38,67	0,01
Overweight	0,66	0,10-4,06	0,6
Obesity	3,78	0,53-26,59	0,1
nulliparous	0,25	0,42-1,49	0,1
Thrombocytopenia	0,62	0,95-4,75	0,6
Noresponseto ASA	9,47	1,64-54,58	0,01

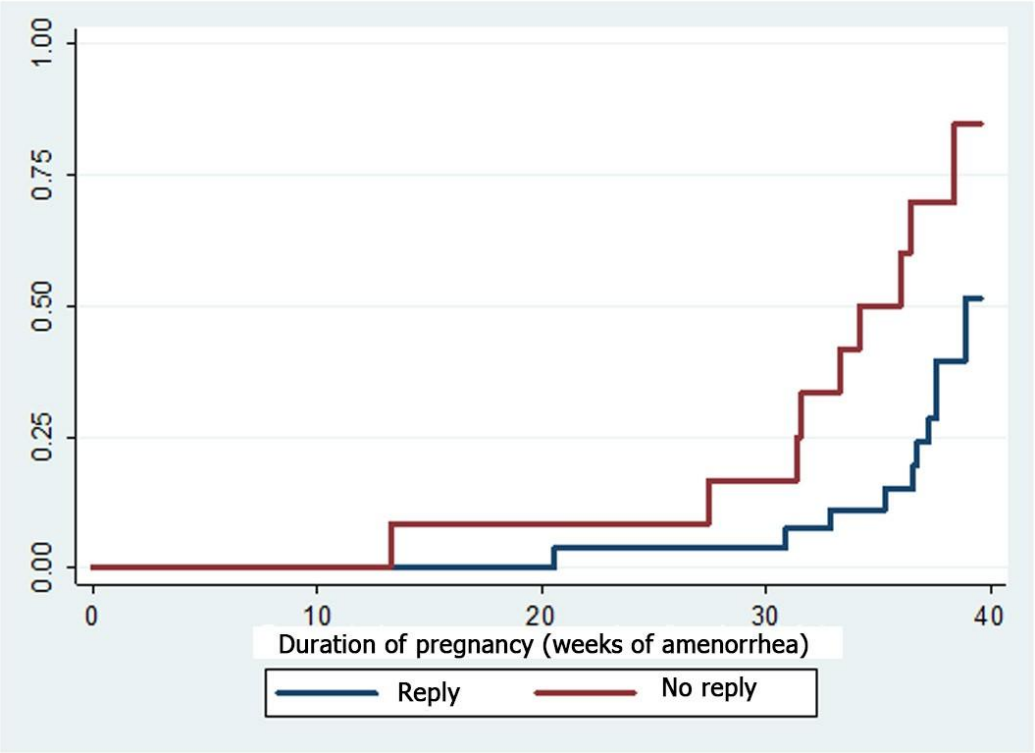

Figure1:Probabilityofobstetricalcomplicationsinnewbornsaccordingtobiologicalresponseto ASA