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When positive studies of novel therapies are subsequently nullified: cumulative meta-analyses in preeclampsia

Abstract

Purpose: The purpose of this study was to examine changes over time in the pooled effect size of randomized, double-blinded, placebo-controlled trials (RCTs) published on the protective effects of antioxidants and low dose aspirin against preeclampsia, and to identify determinants that may affect such changes.

Methods: Two recently published meta-analyses of RCTs examining the effects of antioxidant treatment or low dose aspirin on the rates of preeclampsia and its adverse effects were used. Chronological, cumulative meta-analyses were conducted to investigate the possibility of a time-dependent effect. The journal's impact factor, citation numbers of each paper, and their sample size, were correlated with the risk ratio (RR) of the study.

Results: The median sample size of positive antioxidant trials (i.e., showing protective effect) was tenfold smaller (median 267) than that of the negative trials (median 2120) ($P = 0.017$). A similar trend was seen for low dose aspirin studies. There was a significant correlation between study size and RR for the effects of antioxidants and low dose aspirin on intrauterine growth restriction (IUGR). There was no correlation between RR and citation number, or between RR and the journal's impact factor for the two therapeutic modalities. For both modalities, the journal's impact factor correlated significantly with the number of citations per year. Cumulative meta-analyses revealed that during the first few years and studies, there was a seeming significant protective effect of antioxidant or aspirin against preeclampsia. For both treatment, the initial protective effects gradually disappeared and nullified by larger, later studies.

Conclusions: Initial studies, often published in high impact factor journals, are cited significantly more times but do not exhibit a higher likelihood of predicting a correct long term answer. Studies with smaller sample sizes are more likely to be biased against the null hypothesis. As such, cumulative meta-analysis is an effective tool in predicting potential bias against the null hypothesis and the need for additional studies.

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Typically, following laboratory and experimental animal investigations, novel therapeutic modalities are introduced to humans through case reports and small non-randomized, prospective studies. These are subsequently followed by randomized, double blinded, placebo-controlled trials (RCTs) that eventually allow researchers to define whether the modality is sufficiently effective and safe relative to an existing gold standard. The lag time between the first published RCT and a decision by the medical and scientific communities to accept or reject a new modality can be relatively long. During this period, scientific communications, through editorials, commentaries, letters to editors and lectures, are vehicles that may convince clinicians to use or not to use the new treatment.

In the area of maternal-fetal medicine, new therapeutic options are few and far between [1]. The ethical challenges inherent in exposing a developing fetus to drugs can cause delays and hesitations among clinicians and drug companies alike. Yet, not treating serious maternal conditions can adversely affect the unborn child [2].

Usually, when clinician-investigators complete an RCT of a novel modality, they attempt to publish the results in high citation high impact journals, as these assure wide professional and public dissemination in addition to increased likelihood of future grant funding and professional promotion [3]; however, in more than a few cases, the first, high impact factor publications suggesting a significantly favorable effect had been followed by negative trials.

The objective of the present study was to examine changes over time in the pooled effect size of randomized clinical trials (RCT) published on the protective effects of antioxidants and low dose aspirin against preeclampsia, a common and serious obstetric complication [4], and identify determinants that may affect these changes.

Methods

Two recently published meta-analyses of RCTs, examining the protective effects of antioxidant treatment and those of low dose aspirin against preeclampsia, were used [5-6]. These meta-analyses were subjected to methodological quality assessment using the assessment of multiple systematic reviews' (AMSTAR) method [7]. AMSTAR is a tool containing an 11 point questionnaire with each point having four possible answers. The AMSTAR quality assessment tool falls into three ranges, High [9-11], Medium [5-8], and Low [0-4] [8]. The quality assessment was applied to the studies in the original meta-analyses to ensure that the studies selected were of good quality and their analyses included appropriate inclusion and exclusion

criteria to avoid clinical and methodological heterogeneity and to control for internal validity.

In both meta-analyses, some papers were "positive" in terms of protective effect (defined as RR below 0.9 as this value suggests a 10% protective effect, which was considered by us to be a reasonable minimum) and some were negative (RR equal to or greater than 0.9). The overall result of the antioxidant meta-analysis was "no protective effect" (negative). In the meta-analysis of low dose aspirin, the overall result was marginally protective. These two meta-analyses were subjected to cumulative meta-analysis and correlation studies.

Cumulative meta-analysis

Cumulative meta-analyses of the selected meta-analyses were conducted (without conducting new meta-analyses and adding new studies) to determine if there was a detectable time-dependent effect. The cumulative analysis route displays results accumulated over time: that is, the second row presents a summary analysis comprising the first two studies; the third row presents a summary analysis comprising the first three studies, and so on through the final row. When the data are arranged by year of publication this shows the effect measure (relative risk) that could have been achieved at any point in time with each new study's arrival; furthermore, the changes in the final conclusion can be examined over time. For the cumulative meta-analyses the Comprehensive Meta Analysis Version 2.0 (Biostat, Englewood NJ) was used. Heterogeneity among studies was assessed using the Q statistic and the I-squared test. If the Q statistic (P value) was <0.05, representing a high degree of variance among the studies analyzed, the results are quantified by I-squared values: I-squared between 25%-50% signified low heterogeneity, between 50%-75% signified moderate heterogeneity and >75% signified high heterogeneity [9]. Publication bias was analyzed using Funnel plots for detecting the presence of gray literature and assessing its impact on the analysis. The number of unpublished studies (K) that were possibly absent from our analysis was determined; if evidence of publication bias occurred (K>0), then adjusted point estimates (RR) were calculated based on the number of omitted studies (K) using the Duval and Tweedie's trim-and-fill method.

Correlation studies

Each journal's impact factor, the citation number of each paper determined using Google Scholar during May 2015, and the journal sample size, were correlated with the RR of the study's primary results.

TABLE 1. Details of the RCTs included in the meta-analysis of antioxidants for preventing preeclampsia [5]

Author [reference]	Year	Sample size	RR	Journal name (IF)	Citation No	Citation per year
Han [20]	1994	100	0.10	Chinese Med J (2.34)	46	2.19
Chappell [21]	1999	283	0.46	Lancet (39.20)	857	53.56
Sharma [22]	2003	100	0.48	Int J GynecolObstet (1.56)	68	5.67
Steyn [23]	2003	200	1.00	J ObstetGynaecol (0.60)	53	4.42
Beazley [24]	2005	251	0.92	Am J ObstetGynecol (3.97)	137	13.70
Poston [25]	2006	1877	0.97	Lancet (39.20)	57	6.33
Rumbold [26]	2006	2395	1.20	NEJM (54.42)	398	44.22
Rumiris [27]	2006	60	0.24	HypertensPreg (1.19)	80	8.89
Spinnato [28]	2007	159	0.88	ObstetGynecol (4.80)	105	13.13
Banerjee [29]	2009	1355	0.99	J ObstetGynecol Res (0.90)	13	2.17
Villar [30]	2009	707	1.03	BJOG (1.56)	113	18.83
McCance [31]	2010	2363	0.81	Lancet (39.20)	55	11.00
Roberts [32]	2010	749	1.07	NEJM (54.40)	201	40.20
Xu [33]	2010	9969	1.04	Am J ObstetGynecol (3.97)	89	17.80
Vadillo [34]	2011	444	0.75	BMJ (16.30)	80	20.00

In the case of antioxidants, the journal's impact factor was also correlated with the quality of the paper, using the Cochrane Collaboration method [10]. This method demonstrates which articles satisfied all quality assessment criteria (no risk of bias) and which articles have not satisfied all quality assessment criteria. Comparison of continuous variables was conducted by the Mann Whitney U test, and correlations between variables, were calculated by the Spearman method.

Results

The two meta-analyses included in this review were subjected to quality assessment, where the total AMSTAR scores were high (where both studies had maximum scores of 11). This suggests that the studies included in the meta-analysis are not subjected to clinical and methodological heterogeneity, which may affect the validity of the overall results. The conducted cumulative meta-analysis did not show statistically significant heterogeneity since all the *P* values of the Q tests were less than 0.05 and the *P* values of the I-square were less than 50%.

Antioxidant studies

The first RCT included in the antioxidant meta-analysis was published in 1994 and the most recent was published in 2011. The sample sizes of the 15 studies included those that ranged between 60-9969 subjects, the journals' citation impact factor ranged between 0.60-54.42, and the number of citations of each paper between 13-857 (between 2.17/year and 53.56/year) (Table 1).

The median sample size of the positive trials (median 267) was tenfold smaller than the sample size of the negative trials (median 2120) ($P=0.017$). There was a significant positive correlation between study size and RR ($\rho=0.74$; $P=0.0016$). There was no significant correlation between RR and citation number ($\rho=0.239$), or between RR and the journal's impact factor ($\rho=0.332$). In contrast, the journal's impact factor correlated significantly with the number of citations per year ($\rho=0.82$; $P=0.00016$). Overall, the impact factor of the journal did not correlate with the quality of the papers as measured by Biberio-Salle *et al.* [5] using the Cochrane Collaboration method. Three studies that fulfilled all quality criteria (25, 26, 30), and had no risk of bias, had a RR of more than

TABLE 2. Details of the RCTs included in the meta-analysis of low dose aspirin for preeclampsia (6)

Author [reference]	Year	RR(Sample size)			Journal name (IF)	Citation No	Citation per year
		Preeclampsia	Preterm birth	IUGR			
Wallenburg [35]	1986	0.07(44)	0.12(44)	0.73(44)	Lancet (39.21)	482	16.62
Benigni [36]	1989		0.38(33)	0.31(33)	NEJM (54.42)	249	9.58
Schiff [37]	1989	0.13(65)	0.31(66)	0.30(65)	NEJM (54.42)	314	12.08
McParland [38]	1990	0.11(100)		1.08(100)	Lancet (39.21)	160	6.40
Vinnika [39]	1993	0.84(197)		0.46(197)	BJOG (3.86)	61	2.77
Caspi [40]	1994	0.19(47)	0.75(47)	0.52(94)	Am J Reprod Immunol (2.67)	9	0.43
CLASP [41]	1994	0.88(7974)	0.90(7974)	0.90(8257)	Lancet (39.21)	72	3.43
Hermida [42]	1997	0.43(100)	0.20(100)	0.50(100)	Hypertension (7.63)	49	2.72
Gallery [43]	1997		0.65(108)		Hypertension Pregnancy (1.41)	20	1.11
MFMU [44]	1998	0.90(2503)	0.93(2503)	1.19(2503)	NEJM (54.42)	467	27.47
Grab [45]	2000	1.43(43)			Ultrasound ObstetGynecol (3.85)	26	1.73
Vainio [46]	2002	0.20(86)		0.33(86)	BJOG (3.86)	151	11.62
Yu [47]	2003	0.95(554)	0.90(554)	0.90(554)	Ultrasound ObstetGynecol (3.85)	100	8.33
Villa [48]	2012	0.72(121)		0.33(121)	BJOG (3.86)	49	16.33
Ayala [49]	2012	0.94(350)	0.38(350)	0.49(350)	ChronobiolInt (2.88)	26	8.67

0.9 (0.97, 1.20 and 1.03), showing no protective effect of antioxidant treatment on pre-eclampsia.

During the first 12 years, in five studies, the cumulative meta-analysis revealed that there was a seeming significant protective effect of antioxidant vs. placebo on the rates of preeclampsia. This effect gradually diminished and was nullified by larger studies by 2006 (Figure 1). After the analysis of the publication bias using the funnel plot, the analysis detected five missing studies (Figure 5) and after incorporating the studies in the analysis the overall results shows a stronger non-protective effect of the antioxidant (RR=0.91 in the original meta-analysis, vs. RR= 1.01 adjusted meta-analysis) (Table 3)

Low dose aspirin studies

The first RCT included in the low dose aspirin meta-analysis was published in 1986 and the most recent was published in 2012. The sample sizes of the 14 studies included those that ranged between 44 and 8257 subjects, the journals' citation impact factor ranged between 1.41 -54.42, and the number of

citations of each paper was between 9-482 (0.43/year-16.62/year) (Table2).

The median sample size of the positive trials measuring IUGR (median 72) was 15 fold smaller than the sample size of the negative trials (median 3019) ($P=0.006$). Similar trends were seen for preterm delivery and rates of preeclampsia (Table 2). There was a trend toward significant positive correlation between study size and RR for IUGR ($\rho=P=0.06$). There was no correlation between RR and citation number, or between RR and the journal's impact factor. In contrast, the journal's impact factor significantly correlated with the number of citations per year ($\rho=0.55$; $P=0.05$).

The cumulative meta-analysis revealed that during the first 8-11 years, there was a significant protective effect of low dose aspirin vs. placebo on the rates of IUGR, prematurity and preeclampsia. This effect gradually diminished and was either nullified or remained only marginally significant; a change which was caused by the inclusion of larger studies starting in 2006 (Figures2-4). After the analysis of the publication bias using a funnel plot five to six missing studies were detected - after incorporating these studies in the three meta-analyses, the

TABLE 3. Duval and Tweedie's Trim and Fill summary data for analysis of publication bias in the two meta-analyses

		Random Effects Model				
		Studies Trimmed	Point Estimate	Lower Limit	Upper Limit	Q-Value
Anti-Oxidant & Preeclampsia	Observed Values		0.91	0.80	1.03	23.55
	Adjusted Values	5	1.01	0.94	1.16	46.05
Low Dose Aspirin & Preeclampsia	Observed Values		0.77	0.62	0.95	19.95
	Adjusted Values	5	0.83	0.64	1.07	35.61
Low Dose Aspirin & Preterm Birth	Observed Values		0.86	0.76	0.98	13.37
	Adjusted Values	5	0.89	0.76	1.05	25.76
Low Dose Aspirin & IUGR	Observed Values		0.80	0.65	0.99	19.00
	Adjusted Values	6	1.93	0.74	1.18	33.97

Test of heterogeneity: $df = 14$ ($P = 0.049$) ; $I^2 = 41.071\%$

FIGURE 1. Cumulative chronological meta-analysis of risk ratio in RCTs investigating the effectiveness of antioxidants for preeclampsia (ref.5)

Test of heterogeneity: $df = 12$ ($P = 0.068$) ; $I^2 = 39.841\%$

FIGURE 2. Cumulative chronological meta-analysis of preeclampsia of RCTs of women at risk of preeclampsia who took either aspirin or placebo (ref.6)

Test of heterogeneity: $df = 9$ ($P = 0.146$) ; $I^2 = 32.676\%$

FIGURE 3. Cumulative chronological meta-analysis of preterm birth of RCTs of women at risk of preeclampsia who took either aspirin or placebo (ref.6)

Test of heterogeneity: $df = 12$ ($P = 0.089$) ; $I^2 = 36.830\%$

FIGURE 4. Cumulative chronological meta-analysis of IUGR sorted by the year of publication for trials of women at risk of preeclampsia who took either aspirin or placebo (ref.6)

FIGURE 5. Publication bias using funnel plot of preeclampsia prevention by antioxidant meta-analysis (open circles are the original studies and closed circles are the added studies during adjustment)

overall results showed marginally higher significance (increase from $RR=0.77$ in the original preeclampsia meta-analysis to $RR=0.83$ in the adjusted meta-analysis, from $RR=0.86$ in the original preterm birth meta-analysis to $RR=0.98$ in the adjusted meta-analysis and from $RR=0.80$ in the original IUGR meta-analysis to $RR=1.93$ in the adjusted meta-analysis) (Table 3).

Discussion

Studies published in high impact factor journals are cited significantly more often. Naturally, this leads physicians to encounter these studies more often and possibly assume that these studies reflect the right clinical answer. Similar to our findings, a recent study has shown that the impact factor predicts 59% of the variation in citations of systematic reviews; however, the distribution of citations was obviously skewed [11]. Importantly, our study shows that high impact factor journals do not exhibit higher likelihood of predicting a correct answer, which implies that a higher citation impact does not translate into a more correct clinical impact. The fact that they are cited substantially more often may create a reporting bias; for example, the second published study by Chappel *et al.* on antioxidants [21] showed a 54% reduction in risk of preeclampsia with antioxidants and exhibited the highest number of citations to date ($n=857$), which may impact clinicians' decision in considering patient care. Yet, this study did not correctly predict the overall effect of antioxidants, which was null.

What are the reasons that first studies often declare a dramatic effect only to be nullified later? Our study offers several plausible explanations. Firstly, as shown by our results, studies with smaller sample sizes are more likely to be biased against the null hypothesis and suggest positive results. Small studies are easier to execute and bring to completion, so naturally they are more likely to be published first. As major journals are prefer to publish novel discoveries, they may be more inclined to accept such papers, and, hence, to be exposed to the risk of bias against the null hypothesis.

Secondly, there is a serious and systematic bias against the null hypothesis in the publication process [12, 13]. We and others have shown that negative studies (i.e. those that do not show a significant effect) are less likely to be submitted by their authors [14], less likely to be accepted for scientific presentations or publication by journals [13, 15], less likely to be quoted by the lay media, [16] and less likely to be cited in the peer review literature [17]. When these effects are combined, a serious bias can be produced, potentially creating a spurious positive effect. As years go by, larger studies are published, and the slow-to-be-accepted negative papers eventually manage to

find their way to PubMed–Medline [18]; hence, the original spurious positive effect may be diluted and finally nullified. This is further shown by the assessment of publication bias, suggesting that there are five to six studies that had not been published and, after incorporating the missing studies, the adjusted overall effect showed further nullification of the signal.

With respect to Impact Factor, it has been and continues to be the leading journal quality indicator despite recognized weaknesses such as the effects of self-citation, review articles, the total number of articles published and English language bias [3]. A recent evaluation of 13 trauma journals concluded that the impact factor of a journal was a poor measure of the clinical relevance of its papers. Specifically, the authors found that high impact journals did not address clinical research in surgery and when they did, there was a delay before such papers were cited [18].

In the case of preeclampsia, the biological plausibility of positive beneficial effects from either antioxidants or low dose aspirin has led to great therapeutic hopes, which can probably explain the enthusiasm that accompanied the first positive studies. With both antioxidants and low dose aspirin, the cumulative chronological meta-analyses revealed that during the first years, there was a seeming significant protective effect on the rates of preeclampsia and its complications. With both modalities, the suggested protective effects gradually disappeared and were either nullified or remained only marginally significant by larger, later studies. Our study highlights the importance of the cumulative meta-analysis as a powerful evaluation tool, which has been used more and more often to decide when additional RCTs are no longer needed therefore suggesting an effect has been proven beyond reasonable doubt [19].

The fact that initial papers in high impact journals did not better predict the clinical utility of antioxidants or low dose aspirin for preeclampsia is consistent with the finding that there were no consistent differences in quality between high impact journals vs. papers in less prestigious journals. This highlights one of the criticisms against the citation impact factor, as it may be informative about the overall quality of the journal but not of the quality of specific papers [11].

In summary, initial studies, which are often published in high citation impact factor journals, are cited significantly more times than papers published in less prestigious journals but do not exhibit higher likelihood of predicting a correct answer. Studies with smaller sample sizes are more likely to be biased against the null hypothesis and, as such, cumulative meta-analysis is an effective tool to predict potential bias against the null hypothesis.

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