sustained handwashing with soap and improved water quality in cholera patient households up to 12 months following the intervention.[8] This result was consistent with findings from Deb and colleagues, who found that delivery of narrow-neck drinking water vessels and chlorine to cholera patient households in slums in Kolkata, India led to significant reductions in cholera infections among household contacts.[9] The high efficacy of these interventions is likely attributed to the WASH interventions reducing the spread of cholera within patient households from infected individuals and from contaminated drinking water.

Given the limited supply of OCV globally and the delay in in achieving vaccine protection conferred by a ring vaccination programme, a more comprehensive targeted package of interventions, beyond vaccine alone, is needed. Integration of an intensive WASH programme targeting cholera patients treated at health facilities and their household contacts with an OCV ring vaccination programme for those living in close proximity to the cholera case presents a promising approach for limiting cholera transmission and reducing the number of cholera infections. This intervention would provide protection against cholera for a high-risk population when they are most susceptible and would deliver OCV to a cholera hotspot where overall vaccine efficacy is likely high.

An intervention combining this type of targeted WASH intervention along with a targeted OCV campaign would require cholera patients to be quickly identified at health facilities, OCV to be readily available, and rapid response teams to be ready to intervene. This means a plan needs to be in place before cholera outbreaks occur. We recommend that cholera-endemic countries determine the feasibility of integrating this approach into their cholera control plans.

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A note on the use of Egger regression in Mendelian randomization studies

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A large number of epidemiological studies use genetic variants as instrumental variables to infer causal relationships.[1,2] For a genetic variant to be a valid instrument in these so-called Mendelian randomization (MR) studies,

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three assumptions need to hold: (i) the genetic variant is associated with the exposure of interest (relevance assumption); (ii) the genetic variants should be independent of all confounders (independence assumption); (iii) the genetic variants only effect the outcome through the exposure of interest (exclusion restriction). Without specific knowledge about the biological mechanisms affected by genetic variants, it is virtually impossible to prove that the exclusion restriction holds for a specific genetic variant.[3] For example, genetic variants may have pleiotropic effects on both the exposure and the outcome through different biological pathways.[4]

Several methods and techniques have been developed to tackle the possible problem of pleiotropy in Mendelian randomization studies. In this journal, Bowden and colleagues recently propose using Egger regression to correct for pleiotropic effects of genetics variants.[5] Using simulations, they show that MR-Egger provides unbiased estimates of causal effects if pleiotropy is balanced (i.e. the direct effects are uniformly distributed around zero). Also in case of directional pleiotropy (i.e. the direct effects are uniformly distributed around a non-zero value), MR-Egger performs well, but only as long as the instrument-exposure and instrument-outcome associations are independent. This so-called 'InSIDE' assumption is a relaxation of the exclusion restriction. MR-Egger produces biased results if the InSIDE assumption does not hold, in particular in a one-sample setting in which values for the instrument-exposure association and the instrument-outcome association are obtained in the same sample. Bowden and colleagues acknowledge this in their appendix: 'We conclude that IV analysis with weak instruments in a one-sample setting is troublesome, and that these difficulties are not resolved by the application of MR-Egger regression'.

Nevertheless, MR-Egger is currently often used in epidemiological studies as a robustness check on results obtained with regular Mendelian randomization analysis without proper discussion whether the InSIDE assumption holds. For example, a recent MR study states:

> We used a second method of Mendelian randomisation, the Egger method, as a sensitivity analysis if the instrumental variables test result was noteworthy. This method is more robust to potential violations of the standard instrumental variable assumptions. (…) so this method is less susceptible to confounding from potentially pleiotropic variants (…).[6]

This is an incorrect use of MR-Egger, and hence the conclusions about the robustness of the findings are unwarranted in this study.

Another recent study derived the exact bias of the IVW and MR-Egger estimators.[7] This study recognizes that in some settings where the InSIDE assumption does not hold, the bias of the MR-Egger estimator can be larger than the bias of the regular inverse variance weighting (IVW) estimator. However, no practical conclusions are drawn from this finding. For the purpose of the present note we draw the following conclusion: the use of MR-Egger as robustness check of IVW estimates is prone to unwarranted conclusions about the causal effect estimate, because in empirical settings the assumption that InSIDE holds is often questionable. We will illustrate this conclusion by showing that in two illustrative analyses by Bowden and colleagues,[5,7] the InSIDE assumption does not seem to hold, and that it is not possible in these examples to evaluate whether the MR-Egger is less biased than the IVW estimator.

Methods

Following Bowden and colleagues, we deal with a Mendelian randomization study with N participants.[5] For each participant i, we measure J genetic variants ($G_{i1},..,G_{iJ}$), a modifiable exposure (X_i) and an outcome (Y_i). The genetic variants are assumed to take values 0, 1, or 2, representing the number of alleles of a biallelic single nucleotide polymorphism (SNP). The confounder U_i is a function of the genetic variants and an independent error term (ε_i^U), but is assumed to be unknown. The exposure X_i is a linear function of the genetic variants, the confounder and an independent error term (ε_i^X). The outcome Y_i is a linear function of the genetic variants, the exposure, the confounders and an independent error term (ε_i^Y). The causal effect of the exposure on the outcome is β. γ_j represents the effect of the instrument on the exposure. The coefficients α_j for each genetic variant j represent the direct effects of the genetic variants on the outcome which are not mediated by the exposure. The total effect of each variant on the outcome comprises the direct effect (α_j), and the indirect effects via the exposure ($\beta\gamma_j$) and the confounder (φ_j). The model described above can be written as:

$$U_i = \sum_{j=1}^{J} \varphi_j G_{ij} + \varepsilon_i^U \quad (1)$$

$$X_i = \sum_{j=1}^{J} \gamma_j G_{ij} + U_i + \varepsilon_i^X \quad (2)$$

$$Y_i = \sum_{j=1}^{J} \alpha_j G_{ij} + \beta X_i + U_i + \varepsilon_i^Y. \quad (3)$$

We denote the estimate for the instrument-exposure association by $\widehat{\gamma}_j$ and the estimate for the instrument-outcome association by $\widehat{\Gamma}_j$. With inverse variance weighting (IVW), an estimate for the causal effect $\widehat{\beta}_j$ is obtained by dividing $\widehat{\Gamma}_j$ by $\widehat{\gamma}_j$. This ratio equals $\beta + (\alpha_j + \varphi_j)/(\gamma_j + \varphi_j)$

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(derivation given in the article by Bowden and colleagues[7]), and hence the bias in the estimation of β is a function of α_j, φ_j and γ_j. With multiple genetic variants, the IVW estimator is a weighted average of the ratio of estimates calculated using each genetic variant in turn. In the article by Bowden and colleagues, the bias of the IVW estimator is derived and is equal to $\sum_{j=1}^{J}\widehat{\gamma}_j^2\ \sigma_{Y_j}^{-2}\ ((\alpha_j+\phi_j)/(\gamma_j+\phi_j))/\sum_{j=1}^{J}\widehat{\gamma}_j^2\ \sigma_{Y_j}^{-2}$, where σ_{Yj} is the standard error in the regression of the outcome on the jth genetic variant.[7] In MR-Egger, the absolute values of $\widehat{\Gamma}_j$ are regressed on the absolute values of $\widehat{\gamma}_j$ in order to estimate β. Furthermore, Bowden and colleagues find that the bias in the estimation of β with MR-Egger equals $(\sigma_\alpha\rho_{\alpha,\gamma}+(1+\beta)\sigma_\varphi\rho_{\varphi,\gamma})/\sigma_\gamma$, where σ denotes the standard deviation of a parameter and ρ the correlation.[7] Hence, in MR-Egger the bias is a function of σ_α, $\rho_{\alpha,\gamma}$, β, σ_φ, $\rho_{\varphi,\gamma}$ and σ_γ (note that MR-Egger requires $\sigma_\gamma>0$; this is called the 'variation in instrument strength' assumption, by Bowden and colleagues[7]).

As long as the InSIDE assumptions holds, the bias in MR-Egger is zero if both the sample size and the number of instruments increase to infinity.[5] Although Bowden and colleagues point to some empirical evidence that may suggest that the InSIDE assumption holds for some traits,[8] in general the assumption is quite strong and–more importantly–very difficult to test, since α_j is typically unknown. Thus, from a practical point of view, it is important to know in which settings the bias of MR-Egger is really smaller than the bias of IVW. That is, when does the following inequality hold?

$$\begin{aligned}|Bias_{MR-Egger}| &= \left|\frac{\sigma_\alpha\rho_{\alpha,\gamma}+(1+\beta)\sigma_\phi\rho_{\phi,\gamma}}{\sigma_\gamma}\right| \\ &\leq \left|\frac{\sum_{j=1}^{J}\widehat{\gamma}_j^2\ \sigma_{Y_j}^{-2}\ \frac{\alpha_j+\phi_j}{\gamma_j+\phi_j}}{\sum_{j=1}^{J}\widehat{\gamma}_j^2\ \sigma_{Y_j}^{-2}}\right| = |Bias_{IVW}|.\end{aligned} \tag{4}$$

Since there are so many unknown parameters in (4), it is hard to assess which of the two biases is the largest in a Mendelian randomization study. At first sight, the left-hand side seems smaller, since the bias is mostly based upon covariances and not on real effect sizes. Yet, to show that this is not necessarily the case, we simplify by considering a model where there is no unobserved confounder. In that case, (4) reduces to:

$$\left|\frac{\sigma_\alpha\rho_{\alpha,\gamma}}{\sigma_\gamma}\right| \leq \left|\frac{\sum_{j=1}^{J}\gamma_j^2\ \sigma_{Y_j}^{-2}\ \frac{\alpha_j}{\gamma_j}}{\sum_{j=1}^{J}\gamma_j^2\ \sigma_{Y_j}^{-2}}\right|. \tag{5}$$

Consider a situation where we have relatively strong instruments that all have approximately similar strength, such that $\gamma_j \sim N(0.4,0.1)$. Let there be some directional pleiotropy with an equal variance that is equal to the instrument variance, such that $\alpha_j \sim N(0.1,0.1)$, and let it be positively correlated with γ_j, such that $\rho_{\alpha,\gamma}=0.3$. Now, the expected bias of the MR-Egger estimate is equal to $0.1\times 0.3/0.1=0.3$ and the expected bias of the IVW estimate is approximately $0.1/0.4=0.25$. Hence, in this setting, the bias of the MR-Egger estimate is larger than the bias of the IVW estimate.

In empirical research settings, it is hard to evaluate whether the IVW estimator is more biased than the MR-Egger estimator. For example, Bowden *et al.*[5] estimate the effect of systolic blood pressure on coronary heart disease risk. With IVW, the effect is estimated to be 0.054 (log odds ratio per 1 mmHg change in blood pressure), and with MR-Egger it is estimated to be 0.015 (same units). In the Appendix (available as Supplementary data at *IJE* online), we show that the approximated correlation between the first stage effects γ and the direct effect α is -0.26. Hence, the InSIDE assumption seems to be violated and this makes it impossible to conclude whether the smaller effect estimate obtained with MR-Egger is due to a smaller true effect β or to a change in the bias part of the MR-Egger estimate. In another study, Bowden and colleagues analyse the causal role of plasma urate concentration on coronary heart disease risk.[7] In the Appendix, we show in this model the approximated correlation between the first stage effects γ and the direct effects α is -0.35. Hence, again it is unclear whether the IVW or the MR-Egger estimate is closer to the true β.

Conclusion

In this note, we showed from a practical point of view that the bias of MR-Egger estimator can be larger than the bias of IVW estimator, depending on the parameters in the model. If the InSIDE assumption does not hold, it is clear that the MR-Egger procedure cannot guarantee an estimate that is less biased than the estimate obtained with IVW. The InSIDE assumption is a relaxation of the exclusion restriction, but it is still a strong assumption in itself. From a practical point of view, this makes it almost impossible in empirical settings to judge whether the IVW or MR-Egger estimator is closer to the real value of the causal effect, because the validity of the InSIDE assumption cannot be tested without knowing the true causal effect. Hence, we conclude that the use of MR-Egger as sole robustness check of IVW estimates is prone to unwarranted conclusions about the causal effect estimate. Of course, MR-Egger regression can be used as a sensitivity analysis for Mendelian randomization, but should be treated as a fallible check and in tandem with other analyses to assess the plausibility of the causal effect estimate.[9]

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We note that in some cases, bias from violations of the InSIDE assumption can be solved by finding a specific subsample for which the first stage effect does not exist (the effect of the instrument on the exposure is zero). In such a subsample, the direct effect of an SNP can be estimated and used to correct the causal effect estimate. A recent study in this journal shows that this strategy is able to produce unbiased estimates.[10]

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Misconceptions on the use of MR-Egger regression and the evaluation of the InSIDE assumption

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In their letter to this journal, Slob *et al.*[1] attempt to derive the bias of the MR-Egger regression[2] estimate for a Mendelian randomization (MR) analysis. They show that its bias can be larger than that of the inverse variance weighted (IVW) estimate when the instrument strength independent of direct effect (InSIDE) assumption is violated, and suggest a method for assessing the magnitude of InSIDE violation in any given data set. Slob *et al.* conclude by cautioning against placing undue reliance on the MR-Egger estimate in practice.

Whereas I agree with the basic sentiment of their letter, I wish to make several minor points of correction and clarification. I must also highlight a major flaw in their argument concerning a test for InSIDE violation, so that it is not subsequently repeated by others.

I would not recommend the use of MR-Egger regression, in its current form, in the 'single sample' setting, that is when genetic associations with the exposure and with the outcome are measured in the same subjects. This viewpoint is put forward in my reply[3] to a recent letter by Hartwig and Davies[4] to the *IJE*.

Slob *et al.*[1] helpfully state that the asymptotic bias of the inverse variance weighted (IVW) and MR-Egger estimates (or equivalently their underlying estimands) has in fact already been derived by Bowden *et al.*,[5] specifically in equations (23) and (24). Unfortunately, the expressions given in Slob *et al.*[1] and referenced to Bowden *et al.*[5] do not match, and I have some concerns as to their validity. For example, the expression given by Slob *et al.* for the bias of the IVW estimate depends on the parameter

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