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Studies on Alzheimer Disease - Metformin Connection - A Brief Review

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

Metformin is the widely prescribed first line oral antidiabetic drug used in diabetes mellatus, type 2 . The global sales turnover of metformin runs into millions of dollars. The Increased risk of metformin (Met) users for developing Alzheimer disease (AD)is reported first in a study conducted in 2011. Since then, the subject has attracted the attention of the researchers as well as the pharmaceutical industry, resulting in a number of studies, both clinical as well as experiments on animals. Confusing results poured in , ranging from confirmation of the risk of AD to protection against developing AD , making the scenario, all the more intriguing . Added to the confusion, is the diversity of various studies as well as the parameters interpreting their results. Of the many clinical trials, some are retrospective cohort studies (Tseng Chin-Hsiao 2019) , case control studies (Imfeld P, et al.) Randomised studies (Hsu CC, et al.), double blind , cross over pilot studies. (Aaron Koenig et al.) and some longitudinal studies (Ng TP, et al.) , besides studies doing meta analysis . Of these studies most of the trials estimate the risk of development of dementia with metformin alone (Tseng Chin-Hsiao 2019) or in comparison with other OHAs (Hsu et al, Cheng et al.) .The other studies studied the effect of metformin on the cognition. (Moore EM, et al.). These trials have different out come measures, (like Hazard ratio, (HR) Odds(OR) ratio, relative risk (RR) etc.) which don't mean one and the same. So the multiplicity of the types of studies and different out- comes with different conclusions will be surely baffling to an average reader who tries to take cognisance of the involved issues. The article attempts to take stock of the overall developments in this regard. The author

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adopted a reader friendly approach which is discussed in the article, at the outset. Finally, it is reiterated that future prospective studies only can resolve the conflict of opinion on the nexus between metformin and Alzheimer's disease.

Keywords: Metformin (Met); Alzheimer's Disease (AD); Minimum Cognitive Insufficiency (MCI); Retrospective Studies Hazard Ratio (HR); Odds Ratio (OR).

1. INTRODUCTION

Metformin is the first line of drug used by millions of type 2 diabetes patients for long periods. when the possible risk of long term use of metformin in diabetics resulting in Alzheimer's is first reported, it has become a matter of public health concern . More important are the interests of pharmaceutical industry, with millions of turnover on metformin sales. The metformin market is fast growing with staggering returns in terms of sales. It is anticipated that global market for metformin registers an average annual growth rate of 7.07% from 145 million $ in 2013 to 178 million $ in 2016. Global Metformin Hydrochloride market size will increase to 380 Million US$ by 2025, from 280 Million US$ in 2018, at a Compound annual growth rate (CAGR) of 4.7% during the forecast period .This has given an impetus for extensive studies , both clinical and animal .There is total confusion with some studies suggesting a risk of AD on long term use of metformin , while the other studies suggested a lowering of the risk of developing AD with long term use of metformin. This article attempts to review the literature regarding the evidence available, in this regard.

When dealing with observational studies, in this case , the study of influence of metformin in the development of AD, several technical terms used may baffle the ordinary reader. Though an exhaustive explanation of the entire terminology is out of scope of this article, nevertheless, some basic idea is given, in Appendix A, Table 2, so that the interested may refer elsewhere for more help. The material on the trial details like, sample size, source of data, type and design of study etc., each of them has bearing on the out come of the trial. Accordingly, the study material is formatted on these lines Further, the outcome measure of each trial is different and so is their significance. Hence a modest briefing is given, regarding these issues in Appendix B, Table 3. Every trial is subject to some inherent 'bias' which effects the credibility of the outcome. Important bias type that the reader comes across during this article are presented in Appendix C, Table 4. The various statistical measures and tests that come across in the article are briefed in Appendix D , Table 5. Information on trials is formatted such way that a comparison of parameters of the various trials can be made by the reader. An attempt is made by the author to make the article 'reader friendly' and to equip the reader to draw his own conclusions on matter on hand0 The information on the metformin -AD nexus is the outcome of ongoing research in two directions.

1) Clinical trials.
2) Studies on animals.

Table 1. Showing various clinical trials and their outcome

Study.	**Conclusion**
1) Tseng Chin-Hsiao 2019.	Reduced risk.
2) The Singapore Longitudinal Aging Study(2014).	Reduced risk.
3) Campbell JM, et al. 2018.	Reduced risk.
4) US veterans cohort study.(2017)	Reduced risk
5) Cheng C, et al (2014)	Reduced incidence.
6) Hsu CC, et al. (2011)	Increased risk
7) Weinberg AMV, et al. (2018).	Increased risk.
8) Imfeld P, et al (2012)	Increased risk.
9) Dr Kuan and Ereshefsk. (2017)	Increased Risk.
10) Australian clinical study (2023)	Impaired cognition.
11) Aaron Koenig et al.	Improved cognition.

2. THE BIO CARD STUDY (2017). [14]

The objective of this study is to evaluate whether a simple clinical index (Bio card index) consisting of questions given to patients and their informants, could predict the onset of symptoms of MCI among cognitively normal individuals. Though it is unrelated to the present context, the innovative search to find biomarkers even before MCI is detected in otherwise normal people. It evaluates the independent role of one of the risk factors of old age.

Two hundred twenty-two participants in the BIOCARD study received a detailed history, physical examination, and neuropsychological testing annually. An index was calculated by including questions about memory problems, depression, age, education, history of cerebrovascular disease risk factors, and brain injury, family history of dementia, and the Mini-Mental State examination score. Cox regression analyses were used to determine if this index score was related to diagnosis of MCI.

The BIOCARD Index score mean for individuals who progressed to MCI was 20.3 (SD=2.9), whereas the score for individuals who remained normal was 24.8 (SD=2.3) ($P<0.001$) [hazard ratio, SE for subsequent diagnosis of MCI=0.75 (0.67 to 0.84); $P<0.001$].

Lower BIOCARD Index score predicted symptoms of MCI several years before the MCI diagnosis. The BIOCARD Index can be easily used in clinics to identify cognitively normal older individuals who are at risk for deterioration.

3. EXPERIMENTAL STUDIES ON ANIMALS

Alzforum website has more extensive listing of research models, with 159 models as of September 3, 2018 (increasing from 124 models on April 7, 2017). These models have been described in detail by Li et al. 2016 [1] and modified on 3-6-2019 by Sapeck Agrawal and Gaithersburg, Exhaustive account of all the animal models is out of scope of this article and the interested can surf the sources cited above. Only, the animal modals used the study of the effect of metformin on diabetic /AD are focused in this article. Transgenic mice and db / db mice are widely used in studying Alzheimer pathology in diabetics and particularly, the latter for testing the effects of metformin on AD.

Animal modals used in alzheimer study

A) *In vivo* modal:

1) Transgenic.
2) Natural modal.
3) Non-transgenic modal
4) Intervention modal

B) *In vitro* modal:

1) Tissue cultures: (Brain slices , induced stem cell cultures) .
2) Cell lines: (Nueroblastoma cell lines).

Explanation of terms used in the animal studies

Trans genic mice: Their DNA bears 90% homology with that of human's. They are produced by modifying the genome by genetic engineering. Further each modal has advantages and did advantages which their in a particular study. For example, APP transgenic mice exhibit Aβ plaques, synaptic loss; exhibit cognitive deficits and behavioural impairment but don't express Tau protein of early onset, familial type of AD.

There are several phenotypes of AD. Accordingly, to test them different transgenic mice are available. for example, AD cases expressing only high Amyloid, APP Transgenic Mice are useful. Phenotypes expressing only Tau protein requires Tau Transgenic Mice.

Whereas phenotypes expressing both Tau protein and amyloid protein the double transgenic mice (Tau/ABB) are used. APP/tau/PSN1 triple transgenic mice exhibit. Slow but severe pathology resembling human AD. There are several modal of transgenic mice-loke.

Non transgenic mice (mice fed on high fat diet) are rarely used to study the AD pathology.

The natural modal like non-human primates and dogs are not preferred because of cost and ethical issues. A few studies based on Nueroblastoma cell lines in vitro are reported in literature.

Knockout gene modal: in these a particular gene is knocked out to study the influence exerted by such gene. Knock out mice are useful for studying AD pathology.

Nueroblastoma cell lines: These modals are popular with some in vitro studies involving DM2 / AD.

The studies on db/db mice (Jiejie Li, Jiao Deng, and Z Zuo): db / db (BKS.Cg-Dock7m+/+Leprdb/J) mice are a common model for type 2 DM. The db / db mouse is a genetically mutated mouse in which leptin receptors do not function properly. These mice develop hyperglycemia and hyperinsulinemia and are obese, polyphagic , polydipsia and polyuria. [14,15] db/db mice develop multiple AD-like biochemical brain changes. impaired cognitive functions, increased phospho-tau and Aβ as well as decreased synaptic proteins. The db/db mice had more tau phosphorylated at S396 and total tau in their hippocampi than their non-diabetic control db+ mice. Activated/phosphorylated c-jun N-terminal kinase (JNK), a tau kinase, was increased in the db/db mouse hippocampus. Metformin attenuated the increase of total tau, phospho-tau and activated JNK.

Some observations from db/db mine studies on DM2/ AD / metformin are summarised below.

1) Metformin attenuated the reduction of synaptophysin, a synaptic protein, in the db/db mouse hippocampus.
2) Metformin did not attenuate the impairments of spatial learning and memory.
3) Long-term hyperglycemia in the db/db mice.
4) Consistent with the glucose results, metformin treatment for 18 weeks did not affect the HbA1c levels.
5) Metformin did not improve the spatial learning and memory as assessed by Barnes maze in this study. This finding is seemingly surprising because metformin attenuated the increase of tau phosphorylation and preserved the expression of synaptophysin.
6) Since hyperglycemia can impair the learning and memory functions. [16] it is possible that hyperglycemia in the db/db mice treated with metformin contributes to the failure for metformin to improve the cognitive functions in these mice.
7) The db/db mice had hyperinsulinemia that was not affected by metformin treatment.
8) The db/db mice had higher serum lactate concentrations than the db+ mice ($P = 0.005$, $t(14) = 3.309$). Treatment with metformin or saline did not affect the increased serum lactate concentrations in the db/db mice.
9) Since hyperglycemia can impair the learning and memory functions Kawasaki et al. [17] it is possible that hyperglycemia in the db/db mice treated with metformin contributes to the failure for metformin to improve the cognitive functions in these mice.
10) In addition, leptin is known to facilitate spatial learning and memory (Oomura et al. [18] and the db/db mice have a defect in leptin signaling.
11) All of these factors may contribute to our findings that metformin improved AD-like biochemical changes in the brain but did not improve the learning and memory impairments assessed by Barnes maze in the db/db mice.
12) Unlike insulin, metformin exerts no effect on Aβ degradation. Glucose deprivation and various tyrphostins.
13) Metformin, at doses that lead to activation of the AMP-activated protein kinase (AMPK), significantly increases the generation of both intracellular and extracellular Aβ species. The effect of metformin on Aβ generation is mediated by transcriptional up-regulation of β-secretase (BACE1), which results in an elevated protein level and increased enzymatic activity.
14) Insulin modulates metabolism of β-amyloid precursor protein (APP) in neurons, decreasing the intracellular accumulation of β-amyloid (Aβ) peptides, which are pivotal in AD pathogenesis. Metformin, at doses that lead to activation of the AMP-activated protein kinase (AMPK), significantly increases the generation of both intracellular and extracellular Aβ species. The effect of metformin on Aβ generation is mediated by transcriptional up-regulation of β-secretase (BACE1), which results in an elevated protein level and increased enzymatic activity.
15) Known inhibitors of insulin-like growth factors/insulin receptor tyrosine kinases, do not modulate the effect of metformin on Aβ. Inhibition of AMP-activated protein kinase (AMPK) by the pharmacological inhibitor Compound C largely suppresses metformin's effect on Aβ generation and *BACE1* transcription, suggesting an AMPK-dependent mechanism. Unlike insulin, metformin exerts no effect on Aβ

degradation. Glucose deprivation and various tyrphostins.

16) Although insulin and metformin display opposing effects on Aβ generation, in combined use, metformin enhances insulin's effect in reducing Aβ levels. Our findings suggest a potentially harmful consequence of this widely prescribed antidiabetic drug when used as a monotherapy in elderly diabetic patients.

The authors observations and comments:

On the clinical trials

The role of statistical evaluation in conflicting results

The various statistical measures used in the studies are briefed in respective tables in the appendices. It is beyond the scope of this article to apply acid tests for truthfulness of the methodologies used. The task is left to the readers, having expertise in this field. The ordinary readers are acquainted with some basics of statistical approaches. However by and large each study elaborates the statistical methods and tests used as well as measures taken to prevent various bias. The p values, standard divisions and confidence limits, where made available are of statistical significance. The Cox regression model of constructing the control group is also an accepted method. Still it is an enigma , at least to the author , that how the 3 Taiwan based studies , apparently well designed and using the same data source ie. NIH insurance records, differ widely as to their results. The researchers (Dr Kuan and Ereshefsk), when questioned by a Medscape media personnel has no comments to offer on this issue. It behoves then, that a deep analysis of these studies and their statistical interpretations is warranted, at least in the opinion of this author , to resolve this riddle.

The authors observations, hence are limited to pointing out the merits and demerits if any and highlighting once again the conclusions of each study.

The Study of Tseng Chin-Hsiao 2019.

This is the late test of all studies on MET vs AD .This a well designed study and the authors took all care for the results to be statestically significant and justified as to how they have overcome the different bias. The study finds a positive and beneficial risk relationship for developing.

Chin-Hsiao et al. in their article, have commented of the following short comings in the earlier studies dealt with above . The author does enter into the judgement on the validity of the remarks.

1) Small sample sizes,
2) Prevalent user bias,
3 Immortal time bias
4) Confounding by indication,
5) Lack of dose-response analysis,
6) and inadequate control group .

The authors has to say the following observations regarding the study of Tseng Chin-Hsiao.

1) The over all and tertile-wise data (see Table) show that the HR is lower in the un -cotrolled group than the controlled group NIH Taiwan covers about 99% of its population, its data is claimed to represent true population, statestically. The controlled cohort is constructed using Cox regression and PR. Whether the variation of HR in both groups for the same tertile period is due an unknown bias introduced by the process of constructing the controlled group , is not known.

2) The study, commenting on the high HR in the first tertile, ascribes it the carry forward risk due to the independent risk factor of obesity and cites a reference which supports the usefulness of met for min on the comorbidity. In other words it is left to be inferred that the lowest HR in the subsequent tertile is due to control of this comorbidity. Now the question is why obese subjects are not excluded from the study as it is known to ban independent risk factor for dementia. It becomes increasingly relevant when new onset diabetics are chosen and those on other OHA are eliminated presumably that these don't influence the outcome ascribable to metformin alone.

3) The study also suggests that the results of the second and third tertile need caution in interpretation, but give any overt radon. On the other hand, a reference is cited immediately before this statement which speaks of confounding bias on cummulative data. Thus the tertilewise data doesn't appears to be fully endorsed by the study group itself.

4) Dose- duration - response_is quoted as showing reduction of risk after continuous use of metformin over a period of 2 yrs. It is also possible that metformin may be doing so by

controlling the hyperglycaemia/ DM2WHICH are themselves independent risk factors. Other OHA were also shown to risk reduction and the mechanism is presumably due to control of DM2.

5) The P values of over all and Tertile-wise HRs both in control cohort and study chart were not shin. Hence their statistical significance is difficult to make out.

6) Though Taiwan data Base is well maintained yet, it is agreed that coding errors are possible at the level of coders which again depends on diagnosis and documentation. Diagnosis and documentation are in general poor in got as well as corporate sectors in India. Coding is done by the billing clerk where instead by the insurer centre companies. The idea is not to understate the accuracy od Diagnosis ,documentation and coding system on which most of the Taiwan based studies are dependent, including the present one. There are no authenticated literature on this mater, as far as the author could surf the net.

The study by Hsu et al: This study has shown together, these 2 OHAs (Met and SU) decrease the risk of dementia in T2DM patients by 35% over 8 years.

Tseng Chin-Hsiao et all. Commented on this study "The study compared the risk of dementia in subgroups of diabetes patients with the use of sulfonylureas only, metformin only a Study by Hsu et al. d sulfonylurea plus metformin to a group of diabetes patients without ever use of any antidiabetic drugs might have included an inappropriate control group without the use of any antidiabetic drugs. Furthermore, prevalent user bias and immortal time bias were not well addressed".

The study of Kuan and Ereshefsky

This study's results are diagonally opposite to the above study. While the Tseng Chin-Hsiao study shows risk reduction and dose duration improvement with respect to metformin, this study finds increased risk of AD and correspondingly increased risk with both increased dose and duration. Both data Base is same, the NIH Taiwan insurance data. Why these opposite results could no be explained.

The study of Aaron Koenig et al. -The sample size is small. Furthermore, post-hoc ASL-MRI computer analyses—demonstrating increases in orbitofrontal metabolism with metformin but not placebo—suggest a potential mechanism of action related to effects on frontal-executive pathways. The orbitofrontal cortex is a key prefrontal region involved in information encoding 37, and decreases in regional metabolism have been observed in individuals with AD38.

Post hoc analysis is called data dredging by critics because the statistical associations that it finds are often spurious. It is not accepted by FDA.

I In the pilot study, no structural changes were seen in the areas of brain relevant to Ad while, the converse is true of the current opinion. American Veterans study, the risk below is down below 75 years , but not in those aged above 75 yrs. This is not explained. It is estimated that incidence in people aged more than 75 is higher than those below 75 yrs.

At the same time, deficit in cognitive function was noted whereas the converse is true as per other studies.

The study of Cheng et al.

This study has another facet of study in. Comparing the relative risks of dementia between TZD and met .The study found that TZD users were at an increased risk compared to meformin in developing dementia.

4. MERITS OF THE STUDY

Dementia rate among SU users. The results should be interpreted with caution given the observational design of the study, and the relatively small number of TZD users.

This study is unique in that we followed a large-scale, population-based geriatric cohort of diabetes-free and dementia-free participants to the onset of diabetes and then to the development of dementia, investigating the associations of late-life diabetes, and types and compliance of antidiabetic medication in relation to dementia.

5. LIMITATION OF STUDY

1) Selection bias (both patient selection bias and physician selection bias). This was explained by the authors as being inherent to the type of the dtudu which in this case is a database rather than a randomised cohort study.

2) Short follow-up: The period of study was considered short with respect to a chronic disease like dementia. But the authors contend that earlier age of onset od dementia in nondiabetics as suggested by earlier studies.

The authors contend that there were other studies which have even lesser follow-up time.

US veteran's study: This study brings out the superiority of metformin over SU in reducing the risk for developing AD, which is in line with the results of the other studies. The another important aspect is that risk increases in people aged above 75 yrs, which , according to the study is statistically significant even after adjustments. This paradox has not been explained.

Wennberg study and Imfeld et al study found an increased risk of dementia with. The use of metformin.

The author's observations on animal studies

The symptomatology of diabetes are faithfully reproduced in db/ db mice. The biochemical lesions like increased B- amyloid and phosphorylated Tau protein are highly expressed in db/db mice. Spatial cognition and learning abilities as seen I cases of AD are also shown to be impaired in db/db mice. So db/db mice is expected to yield good information on met for min role in AD. Though , meteor min is shown to reduce biochemical changes in brain ad mentioned above , and some cognitive defect is improved by met for min, there are certain differences of met for min action in humans and db/db mice. Meteor min reduces hyperglycaemia, HbA1C levels and hyperinsulinemia and serum lactate in human being , it is the converse wet db/db mice. It is known that the enlisted effects of met for min are due to insulin sensitising effects of met for min in human being. Failure to accomplish these effects in db/db mice raises the question as to met for min acts as insulin sensitised in db/ db mice. The achieving of biocidal profile improvement in spite of not having insulin sensitising effect of met for min in db/db mice raises the question whether insulin sensitising effect of met for min is the mechanism behind such changes. Further, improvement in some cognitive function and learning in db/db mice suggests that some other mechanism other than insulin sensitising action of met for min may be operating. It follows that the biochemical and the cognitive function may be achieved by different mechanisms of met for min.

6. CONCLUSION

When two extreme opinions exist , the truth must be somewhere in the middle- as the saying goes. The real answer to the question can be given by a future prospective study only.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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APPENDICES

Appendix - A Table 1.

Table – 2

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Explain nation of the technical terms pertaining to studies described.

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Prospective vs. Retrospective Study: In a retrospective cohort study, the group of interest, already has the disease/outcome. In a prospective cohort study, the group does not have the disease/outcome, although some participants usually have high risk factor The retrospective studies (RCT) are considered as 'quick and dirty' but their value as pilot studies for rare disease can not be ignored . There are 3 general types of retrospective study: case report, case series, and case-control study. Prospective studies are a gold standard but they are time consuming and laborious. Even the studies quoted in this article concede this point.

The outcome measure of RCT is relative risk (RR)

Case control studies: They are retrospective studies.. They clearly define two groups at the star: one with the disease and one without the disease. They look back to assess whether there is a statistically significant difference in the rates of exposure to a defined risk factor between the groups. This can suggest associations between the risk factor and development of the disease although no definitive causality can be drawn.

The outcome measures is odds ratio (OR).

Cohort studies: Cohort studies include two groups (one with exposure and the other without exposure) that are identical EXCEPT for their exposure status. If a significant number of participants are not followed up (lost, death, dropped out) there may be attrition bias – a significant difference between the groups of those that did not complete the study.

Randomizer study: The allotment of members to a group or within groups those who are administered drug or placebo is selected by chance like tossing a coin.

Single and double blind study: On single blind study a member is not aware to which group he is allotted or whether he is administered drug or placebo. But the administrator knows. In the double blind study, both the subject and the administrator are not aware of these facts.

Crossover over study – over time, each participant receives (or does not receive) an intervention in a random sequence.

Longitudinal study: A study carried over a long time.

Cross sectional study: Type of observational study that analyzes data from a population, or a representative subset, at a specific point in time—that is, cross-sectional data.

Appendix - B Table 3. Outcome measures and their significance of the trials

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Relative Risk Ratio (RR)

Is the ratio of the probability of an outcome in an exposed group to the probability of an outcome in an unexposed group. It is computed as, where is the incidence in the exposed group, and is the incidence in the unexposed group.

Significance:

RR = 1 means that exposure does not affect the outcome;
RR < 1 means that the risk of the outcome is decreased by the exposure;
RR > 1 means that the risk of the outcome is increased by the exposure

The hazard ratio is an estimate of the ratio of the hazard rate in the treated versus the control group. The hazard rate is the probability that if the event in question has not already occurred, it will occur in the next time interval, divided by the length of that interval.

Significance of HR: Hazard ratio of 1 means lack of association, a hazard ratio greater than 1 suggests an increased risk, and a hazard ratio below 1 suggests a smaller risk.

Odds ratio (OR): Is a statistic that quantifies the strength of the association between two events, A and B. The odds ratio is defined as the ratio of the odds of A in the presence of B and the odds of A in the absence of B, or equivalently (due to symmetry), the ratio of the odds of B in the presence of A and the odds of B in the absence of A.

Significance of OR: Two events are independent if and only if the OR equals 1: the odds of one event are the same in either the presence or absence of the other event. If the OR is greater than 1, then A and B are associated (correlated) in the sense that, compared to the absence of B, the presence of B raises the odds of A, and symmetrically the presence of A raises the odds of B. Conversely, if the OR is less than 1, then A and B are negatively correlated, and the presence of one event reduces the odds of the other event.

HR vs RR and OR: Hazard ratios differ from relative risks and odds ratios in that RRs and ORs are cumulative over an entire study, using a defined endpoint, while HRs represent instantaneous risk over the study time period.

Appendix - C Table. 4

Important types of 'bias' encountered in the article.

Neyman Prevalence Bias is a selection biaswhere the very sick or very well (or both) are erroneously excluded from a study. The bias("error") in your results can be skewed in two directions: Excluding patients who have died will make conditions look less severe.

Selection bias is the bias introduced by the selection of individuals, groups or data for analysis in such a way that proper randomization is not achieved, thereby ensuring that the sample obtained is not representative of the population intended to be analysed.

Confounding by indication: A distortion that modifies an association between an exposure and an outcome, caused by the presence of an indication for the exposure that is the true cause of the outcome.

Immortal time bias in Pharmaco-epidemiology refers to a period of cohort follow-up time during which death (or an outcome that determines end of follow-upo cannot occur.

Inappropriate assignment of treatment status and follow-up time may introduce immortal time bias by including the so- immortal time (the follow-up period during which the outcome cannot happen) in the calculation of the follow-up period [30].

APPENDIX D. Table 5 .Some statistical tests and procedures:

The p-value

It is, for a given statistical model, the worst-case probability that, when the null hypothesis is true, the statistical summary would be greater than or equal to the actual observed results.

Most authors refer to statistically significant as P < 0.05 and statistically highly significant as P < 0.001 (less than one in a thousand chance of being wrong).

The confidence interval (cI)

It is a range of values, above and below a finding, in which the actual value is likely to fall. The confidence interval represents the accuracy or precision of an estimate. if the significance level is 0.05, , the corresponding confidence level is 95%.

A 95% confidence interval (CI) is a range of values that you can be95% certain contains the truemean of the population. With large samples, you know that mean with much more precision than you do with a small sample, so the confidence interval is quite narrow when computed from a large sample.

Confidence limits are the numbers at the upper and lower end of a confidence interval; for example, if your mean is 7.4 with confidence limits of 5.4 and 9.4, your confidence interval is 5.4 to 9.4. Most people use 95% confidence limits, although you could use other values.

Standard deviation is a number used to tell how measurements for a group are spread out from the average (mean), or expected value. A low standard deviation means that most of the numbers are close to the average. A high standard deviation means that the numbers are more spread out.

Student'st test: A t-test is a type of inferential statistic used to determine if there is a significant difference between the means of two groups, which may be related in certain features. A t-test is used as a. hypothesis testing tool, which allows testing of an assumption applicable to a population.

The chi-squared test: It is used to determine whether there is a significant difference between the expected frequencies and the observed frequencies in one or more categories. ... A chi-squared test can be used to attempt rejection of the null hypothesis that the data are independent.

Cox regression (or proportional hazards regression) is method for investigating the effect of several variables upon the time a specified event takes to happen. In the context of an outcome such as death this is known as Cox regression for survival analysis.

Analysis of covariance is used to test the main and interaction effects of categorical variables on a continuous dependent variable, controlling for the effects of selected other continuous variables, which co-vary with the dependent. The control variables are called the "covariates."

Multivariate analysis of covariance is an extension of analysis of covariance methods to cover cases where there is more than one dependent variable and where the control of concomitant continuous independent variables – covariates – is required.

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1) HSU CC, et al. (2011) [1]

Aim of the study: to estimate association between dementia, DM, and OHAs. Using DID and HR (hazard ratios), calculated with respect to the test and control group.

Type of study: Randomised Cohort trial.

Data Base: Representative cohort of 800,000 from Taiwan's National Health Insurance database.

Duration of study: January 1, 2000, December 31, 2007.

Inclusion criteria: Age -50 years or older, dementia free.

Exclusions: Vascular dementias.

Sample size: Total - n = 127,209

Those with absent inclusion criteria

(Control group)

n = 101,816

Those with presence of inclusion criteria

(Test group)

(n = 25,393).

Clinical Assessment: Dementia was ascertained by ICD9-CM or A-code. Dementia incidence densities (DID) and fully adjusted Cox proportional hazard models were used to estimate association between dementia, DM, and OHA.

The observations: DID per 10,000 person-years was markedly increased with DM without medication, compared to DM free subjects (119 versus 46). Using non-DM as reference,

The adjusted hazard ratios (HRs) (95% confidence interval) for DM without and with OA were 2.41 (2.17–2.66) and 1.62 (1.49–1.77), respectively. For T2DM, compared with no medication.

sulfonylureas alone reduced the HR from 1 to 0.85 (0.71–1.01) metformin alone to 0.76 (0.58–0.98), while with combined oral therapy the HR was 0.65 (0.56–0.74). Together, these 2 OHAs decrease the risk of dementia in T2DM patients by 35% over 8 years.

The conclusions; T2DM increases the risk of dementia more than 2-fold, non-stroke related dementias were found to be decreased in DM with sulfonylurea and metformin therapy.

2) Tseng Chin-Hsiao 2019 [2]

Aim of the study: To determine dementia risk associated with metformin use in type 2 diabetes.

Type of the study: Retrospective population based cohort study.

Data Base: Data base of the Taiwan's National Health Insurance. Investigated patients by using the reimbursement.

Duration of the study: new-onset diabetes during 1999-2005 and we're followed up until December 31, 2011.

Sample Size: Unmatched cohort:

147,729 users of metformin

15,676 non – users of met forming.

Matched-pair cohort

15,676 users

15,676 non- users

(The Cohort was created by propensity score (PS). Hazard ratios were estimated by Cox regression incorporated with the inverse probability of treatment weighting using PS.

Results:

In the unmatched cohort, 71- users and 3943 users developed dementia.

The respective incidence is1029.20and 570.03 per 100,000 person-years.The overall hazard ratio was 0.550 in the unmatched cohort.

The matched cohort showed an overall hazard ratio of 0.707.

Table 6. Tabulation of over all and tertilewise data of study and control groups

	1ST *	2nd. * -	3rd *	overall HR
	(<27.0 m)	(27- 58.1 m)	>58.1 m	
A	0.975. -	0.554 -	0.286.	0.707
B	1.279 -	0.704 -	0.387.	0.550

A = unmatched cohort : B = matched cohort; m = month

. Tertile ; (Any of the two points that divide an ordered distribution into three parts, each containing a third of th population. (statistics)*

Findings of the HR in unmatched and matched cohort groups as well as overall HR of the two groups are shown comparatively in the table. In tertile analyses**,** the hazard ratios suggested a reduced risk in a dose-response pattern. Patients who had used metformin for more than 2 years in the second and third tertiles consistently showed a significantly reduced risk. For the first tertile, the risk was neutral in the unmatched cohort analysis but was slightly higher with a significant *p*-value in the matched cohort. interesting that patients in the first tertile of short-term metformin use showed a significantly higher risk of dementia in the matched cohort analysis , because obesity is one of the major risk factors associated with an increased risk of dementia. [3] and metformin is strongly indicated for diabetes patients with obesity , the increased risk in the first tertile might have been carried over from patients with obesity who were first initiated with metformin treatment. worthy to point out that immortal time might be introduced when the cumulative duration increased because the patients should have lived long enough without development of dementia up to the time of the cumulative duration. Lévesque *et al.* pointed out that there is a “direct relation between the immortal period and the magnitude of the bias”. [4] Therefore, the magnitude of the hazard ratios in the second and third tertiles should be interpreted more cautiously and the dose-response effect could not be fully clarified in the present study.

Conclusion: Metformin use is associated with a reduced dementia.

3) Wennberg AMV, et al. (2018). [5]

Based on cognitive test performance and mild cognitive impairment (MCI) diagnosis among 508 cognitively unimpaired at baseline type II diabetics enrolled in the Mayo Clinic Study of Aging. propensity scores are created to adjust for treatment effects. They used multivariate linear and logistic regression models to investigate the cross-sectional association between treatment type and cognitive test z scores, respectively. Mixed effects models and competing risk regression models were used to determine the longitudinal association between treatment type and change in cognitive test z scores and risk of developing incident MCI.

They did not observe an association between metformin use and cognitive test performance over time (median = 3.7-year follow-up). Metformin was associated with an increased risk of MCI (sub hazard ratio (SHR) = 2.75; 95% CI = 1.64, 4.63, P < .001)..03 per 100,000 person-years.

4) Imfeld P, et al (2012). [6]

Aim of the study: To find out the risk of developing AD in diabetic patients treated with metformin or other antidiabetic drugs (OHA & insulin).

Type: population based Case-control study.

Data base: The United Kingdom-based General Practice Research Database (GPRD),

Sample size: 7086

Controls: Equal number of matched controls without dementia. Matching criteria were demographic factors and years of history in the database.

Duration of study: 1998 to 2008

Results

As compared with nonusers, long-term users of 60 or more metformin prescriptions were at greater risk of developing AD adjusted (AOR) = 1.71,
(95% CI = 1.12-2.60).

No consistent trends were seen with increasing number of prescriptions. Long-term use of other antidiabetic drugs such as sulfonylureas (AOR = 1.01, 95% CI = 0.72-1.42), thiazolidinediones (AOR = 0.87, 95% CI = 0.31-2.40), or insulin (AOR = 1.01, 95% CI = 0.58-1.73) was not related to an altered risk of developing AD.

Conclusion

1) Long-term use of sulfonylureas, thiazolidinediones , or insulin was not associated with an altered risk of developing AD. 2)There was a suggestion of a slightly higher riskf AD in long-term users of metformin.

5) The Singapore Longitudinal Aging Study. [7]

Ng TP, et al. 2014

Studied 365 persons aged 55 and over in the population-based Singapore Longitudinal Aging Study with diabetes who were followed up over 4 years. The odds ratios (OR) of association of metformin use (n = 204) versus non-use (n = 161) with cognitive impairment (Mini-Mental State Exam ≤ 23), and by duration: up to 6 years (n = 114) and more than 6 years (n = 90) were evaluated in cross-sectional and longitudinal multivariate analyses. metformin use showed a significant inverse association with cognitive impairment in longitudinal analysis (OR = 0.49, 95% CI 0.25-0.95). Metformin use showed significant linear trends of association across duration of use in cross-sectional and longitudinal analyses (p = 0.018 and p = 0.002, respectively), with use for more than 6 years significantly associated with lowest risk of cognitive impairment in both cross-sectional analysis (OR = 0.30, 95% CI 0.11-0.80) and in longitudinal analysis (OR = 0.27, 95% CI 0.12-0.60).

Conclusion: Among individuals with diabetes, long-term treatment with metformin may reduce the risk of cognitive decline. (odds ratio 0.49, 95% confidence interval 0.25-0.95).76.

6) The Australian clinical study: [8]

Moore EM, et al. (2013)

Research design and methods : Participants were recruited from the Primary Research in Memory (PRIME) clinics study, the Australian Imaging, Biomarkers and Lifestyle (AIBL) study of aging, and the Barwon region of south-eastern Australia. Patients with Alzheimer disease (AD) (n=480) or mild cognitive impairment (n=187) and those who were cognitively intact (n=687) were included; patients with stroke or with neurodegenerative diseases other than AD were excluded. Subgroup analyses were performed for participants who had either type 2 diabetes (n=104) or impaired glucose tolerance (n=22).

Results: Participants with diabetes (n=126) had worse cognitive performance than participants who did not have diabetes (n=1,228; adjusted odds ratio 1.51 [95% CI 1.03-2.21]). Among participants with diabetes, worse cognitive performance was associated with metformin use (2.23 [1.05-4.75]). 64, 4.63, P <.001.

6) Campbell JM, et al. (2018)[9]

The initial search resulted in 862 citations from which 14 studies (seven cohort, four cross-sectional, two RCTs, and one case control) were included These reaches did Meta-analysis of three studies which showed that cognitive impairment was significantly less prevalent in diabetics on metformin (Odds ratio=0.55, 95% CI 0.38 to 0.78), while six studies showed that dementia incidence was also significantly reduced (Hazard ratio=0.76, 95% CI 0.39 to 0.88). Mini-Mental State Examination scores were not significantly affected by metformin-use, although both RCTs showed that metformin had a neuroprotective effect compared to placebo. Some studies found negative or neutral effects for metformin use by people with diabetes; the potential mechanism of metformin-induced dementia is perhaps due to vitamin B12 deficiency .The authors concluded that Metformin should continue to be used as a first line therapy for diabetes in patients at risk of developing dementia or Alzheimer's disease. The use of metformin by individuals without diabetes for the prevention of dementia is not supported by the available evidence.

7) US veterans cohort study: (2017) . [10]

Type of study: cohort study.

Subjects: US veterans with DM2 who were new users of either metformin or a sulfonylurea and who did not have dementia.

age. Criteria ≥65,
mean age - 73.5;

Sample Size: 17,200 (metformin group)

11,440 (sulfonylurea group)

Treatment period: 2 years.

Follow-up period: 5 years

Results: Total dementia cases- 4906
metformin group -- 2177
(12.7%)
sulfonylurea group - 2729
(23.9%)

The over all crude HR for metformin vs sulphanylureas - 0.67 (95% CI, 0.61-0.73; P <.001) and 0.78 (95% CI, 0.72-0.83; P <.001) in patients age <75 and ≥75, respectively.

After adjustment, the results continued to be statistically significant in veterans age <75 (HR 0.89; 95% CI, 0.79-0.99; P=.033) but not in veterans ≥75 (HR 0.96; 95% CI, 0.87-1.05; P=.332).

Conclusion: metformin was associated with a lower risk of subsequent dementia than sulfonylurea use in veterans <75 years of age.

8) Aaron Koenig, et al - Pilot study on metformin and AD . [11]

Type of study: double blind, crossover pilot trial.

Duration of study: 8 weeks duration.

Type of approach: It employed a multidimensional biomarker panel to explore the effects of metformin in MCI and early dementia due to AD. Plasma, CSF, neuroimaging, and cognitive data.

Sample size: 20 subjects aged between 50 to 80 years

Inclusion and exclusion criteria.

Subjects are neither diabetics or pre-diabetics (fasting -blood glucose <110 or HgbA1c < 6.0) but mild cognitive insufficiency (MCI) screened by CDR-Global ≤ 1.0), screening Mini-Mental State Examination > 19, at least one positive biomarker consistent with AD (e.g. CSF analysis, FDG-PET, amyloid scan).

Study details: Subjects were randomized 1:1 to receive metformin (2000 mg/d) for 8 weeks followed by placebo for 8 weeks or vice versa .The dosage titration and administration schedule was as follows: metformin 500 mg (or placebo) by mouth daily for 1 week, then daily dose (in divided doses) increased by 500 mg per week until a maximum of 2000 mg/d (1000 mg twice daily) was titrated depending on tolerability. reached.

Clinical assessment;

The Clinical Dementia Rating (CDR) scale was performed at screening and week 0 to measure degree of functional impairment, including ratings on degrees of impairment in memory, orientation, judgment and problem solving, community affairs, home and hobbies, and personal care.

Cognitive and functional testing was performed at weeks 0, 8, and 16. Cognitive testing included paper-and-pencil (*Cognitive Subscale of the Alzheimer's Disease Assessment Scale* [ADAS-Cog][31]) as well as computerized (*Cambridge Neuropsychological Test Automated Battery* [CANTAB][32]) assessments.

Executive functioning (Trails-B [TMT-B] time, score on backwards Digit Span [DS]), attention (score on forward DS, percent correct on simultaneous DMS), language (Boston Naming Test total), and motor speed (CANTAB Reaction Time [RTI], TMT-A time). The *Geriatric Depression Scale* (GDS) was administered at weeks 0, 8, and 16 to screen for concomitant depressive symptoms that could affect cognition or trial participation[33].

Laboratory assessment:

1) Neuro - imaging by ASL MRI

Metformin was associated with improved executive functioning, and trends suggested improvement in learning/memory and attention. No significant changes in cerebral blood flow (CBF) were observed, though post-hoc completer analyses suggested an increase in orbitofrontal CBF with metformin exposure.

2) CSF analysis: Measurable amounts of metformin was observed in the CSF hinting some role metformin may play on AD.

Study conclusions: ASL MRI studies did not show much changes in the areas of brain supposed to be involved in AD. CSF analysis showed measurable quantities of metformin but no effect on biomarkers was detected. Since metformin crosses BBB, it may have some implications on the progression of AD. The executive functioning during treatment with metformin but not placebo, and trends suggested improved learning, memory, and attentional abilities during metformin treatment as well. These positive findings, despite the limited sample size and relatively short length of the trial, are promising and warrant further exploration. Furthermore, post-hoc ASL-MRI completer analyses—demonstrating increases in orbitofrontal metabolism with metformin but not placebo—suggest a potential mechanism of action related to effects on frontal-executive pathways. The orbitofrontal

cortex is a key prefrontal region involved in information encoding[37], and decreases in regional metabolism have been observed in individuals with AD[38].

10) Cheng, et al (2014). [12]

Type of study: Population based chart study.

Sample size: 67,731

Data source: NIH insure nice , Taiwan.

Inclusion criteria: Participants who were non-demented, nondiabetic, aged 65 or over.

Duration of the study: from January 2004 to December 2009.

Results

The hazard ratio for dementia diagnosis in the new-onset TZD participants compared with the non-TZD participants was 1.56 (95%CI: 1.39–2.18). The relative rate of dementia was 5.31 (95% CI: 1.89–14.96) for participants taking thiazolidinediones (n = 28) and 1.22 (95% CI: 0.78–1.91) for those taking sulfonylureas (n = 796) compared to those taking metformin (n= 1,033). The risk of dementia was higher in ever (n = 841) versus never users (n = 4,579) of thiazolidinediones : 1.44 (95% CI: 1.12–1.86).

Conclusions

Diabetes is associated with an increased risk of dementia. The risk deceased in the participants who took sulfonylureas or metformin rather than thiazolidinediones for a longer period.

11) Dr Kuan and Ereshefsky: [13]

The results were presented at AD/PD 2017: The 13th International Conference on Alzheimer's and Parkinson's Diseases by Yi-Chun Kua.

Aim: To find out the risk of AD and PD in met users.

Type of study: Database cohot study

Data Base: NIH Taiwan insurance statestics

Duration; 2000 to 2012

Sample size.: (N) 9300

Met users..... 4651

Nonetheless users 4651

Results: The risk for Parkinsonism n disease (PD) or s ; dementia was more than double during a 12-year period for those who took metformin vs those who did not — even after adjusting for multiple confounders.In addition, outcome risks increased progressively with higher dosage and longer duration of treatment.

Table 7.

HR : Met users and non users
All-cause dementia11.5 vs 6.71.66 (1.35 - 2.04)
Alzheimer's dementia1.64 vs 0.832.13 (1.20 - 3.79)
Vascular dementia1.64 vs 0.692.30 (1.25)

Table 8.

Duration of use of metformin vs HR:

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180 - 300 d11.41.79 (1.32 - 2.43)
300 - 400 d10.41.61 (1.21 - 2.16)
≥400 d20.62.84 (2.12)

Table 9.

Metformin dose-wise rusk :

(For all-cause dementia);
<130 g 9 .971.22 (0.90 - 1.67)
130 - 240 g 12.01.61 (1.19 - 2.17)

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle3.com/review-history/50105