IJPSR (2019), Volume 10, Issue 7 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 11 May 2019; received in revised form, 14 June 2019; accepted, 17 June 2019; published 01 July 2019

COMPARISON OF DIFFERENT CLASSES OF ORAL ANTIDIABETIC DRUGS IN COMBINATION WITH METFORMIN ON COGNITIVE FUNCTIONS

N. K. Nair [1], N. Vidhya [1], M. D. Mingate [1] and P. R. A. Vijayakumar [*2]

Department of Pharmacy Practice [1], Department of Pharmacology [2], JSS College of Pharmacy, Rocklands, (JSS Academy of Higher Education & Research), Ooty - 643001, Tamil Nadu, India.

Keywords:

Type 2 Diabetes mellitus, Cognitive function, Oral Hypoglycaemic agents, MMSE

Correspondence to Author:
P. R. A. Vijayakumar

Department of Pharmacology,
JSS College of Pharmacy, Rocklands, (JSS Academy of Higher Education & Research), Ooty - 643001, Tamil Nadu, India.

E-mail: ootyanand2004@gmail.com

ABSTRACT: Type 2 diabetes mellitus is associated with cognitive impairment. The antidiabetic treatment found to delays the impairment. However, the benefit is due to antidiabetic drugs, or control of blood sugar is debatable. Most of the drug-based studies used monotherapy, which contradicts the clinical practice. This study aims to assess cognitive function in type-2 diabetes mellitus patients on metformin and other oral hypoglycaemic agent combination treatment. The cross-sectional study included 325 type 2 diabetes patients based on inclusion and exclusion criteria. They were categorized based on a combination of oral antidiabetics. Socio-demographic and medication data were obtained using medical records and personal interviews. Mini-Mental State Examination Scale-Tamil version (MMSE) was applied to measure the cognitive functions. Aged patients showed a reduced MMSE score of 18.18 ± 1.34. Increased duration of diabetes, >15 years, had the least MMSE score of 21.36 ± 1.37. Metformin-DPP-IV inhibitors combination found to benefit the cognition MMSE score = 29.11 ± 0.19 (P<0.001), compared to alpha glucosidase-metformin and thiazolidinedione- metformin combinations. A similar observation was found in individual domains of cognition as well. Education found to be protective of cognitive impairment. The stability in the blood glucose control and inherent mechanisms of sitagliptin might help in delaying cognitive impairment among type 2 diabetes. Sitagliptin-Metformin combination found to have better cognitive scores compared to other metformin-oral antidiabetic combinations.

INTRODUCTION: Diabetes mellitus, an endocrine disease, if left untreated results in mortality. Diabetes mellitus affects approximately 387 million people worldwide, and this number is expected to rise by 55% to about 592 million people by 2030 [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.10(7).3455-60

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.10(7).3455-60

Poor cognition, impaired brain structural integrity, and function have been associated with uncontrolled diabetes mellitus [2]. Notably, type 2 diabetes mellitus affects executive function, perceptual speed, complex motor functioning, semantic memory, and immediate recall [3, 4]. Age of onset of diabetes mellitus as well as the duration of the disease affect the progression of cognitive decline but also confounded by other co-morbid vascular or non-vascular variables [4]. Vascular diseases in midlife are associated with late-life cognitive impairment [5]. It is speculated that the pharmacological management of diabetes decreases the rate of cognitive decline [6].

However, it remains uncertain whether the beneficial effects are due to glycaemic control or the neuroprotective effects of antidiabetic drugs [7]. For instance, several studies failed to confirm a beneficial cognitive function with improved glycaemic control [8, 9].

Some studies claim an association between metformin and impaired cognitive performance [10, 11]. Moreover, findings from a study that used rosiglitazone to control diabetes mellitus may offer a strategy for the prevention of cognitive decline associated with AD [12]. Also, dipeptidyl-peptidase-4 enzymes inhibitors when administered to insulin-resistant rats, increase peripheral insulin sensitivity, and decrease brain dysfunction [13]. However, Farlow failed to find any clinical significance in the use of antidiabetic drugs to delay the progression of MCI to AD [14]. All these studies fail to come to a definite conclusion regarding the benefits of antidiabetic drugs in preventing cognitive decline.

In a clinical setting, diabetes mellitus is treated with a combination of drugs. However, most of the studies mentioned above are based on single pharmaceutical therapeutic agents. Therefore, the present study aims are; to assess cognitive function in type-2 diabetes mellitus patients who were on treatment with combinations of metformin with different classes of oral hypoglycaemic agents and to analyze if antidiabetic drugs influence cognitive function.

MATERIALS AND METHODS:

Study Design: This study is a cross-sectional study to examine the association of different oral anti-diabetic combination therapy on cognitive functions. The study was conducted in diabetes outpatient department in the Govt. Headquarters Hospital, Ooty, The Nilgiris for six months between 1st October 2015 and 30th March 2016. The protocol was approved by the JSS Ethics Committee, Ooty (JSSCP, OOTY/IEC/09/2015-16).

TABLE 1: INCLUSION AND EXCLUSION CRITERIA OF THE PARTICIPANT

Inclusion Criteria	Exclusion Criteria
Type II Diabetes mellitus patients with or without co-morbidities	Type I Diabetes patients, CNS Disorders, previous history of head injury
On combination therapy of oral anti-diabetic agents for at least six months	Patients with less than 6 months duration of disease, on Insulin therapy, Neurotic drugs, Psychiatric medicines
Patients above 25 years of age and willing to participate	Pregnant and lactating women

Participants: A total of 400 adults with diabetes mellitus were screened based on prefixed inclusion and exclusion criteria (see **Table 1**). Participants were excluded, if they had type 1 diabetes mellitus (n = 3), Epilepsy (n = 5), Parkinson's disease (n = 2), history of Stroke (n = 8), history of head injury (n = 4), were on Psychiatric medication (n = 3), on Insulin therapy (n = 22), and on single oral antidiabetic medication (n = 21), newly diagnosed type 2 diabetes (n = 7) patients who are pregnant (n = 4) & lactating (n = 6) were also excluded.

After exclusions, 325 types 2 diabetes mellitus patients, between the ages of 25–-5 years were included in the study. An informed consent form was obtained from each of the patients.

Determination of Type 2 Diabetes Mellitus: Diabetes mellitus was determined by a self-report by the patient or by the use of antidiabetic medications. The duration of type 2 diabetes mellitus and type antidiabetic medication used were obtained from medical history and medical records.

The duration of the disease was then categorized into; <5 years (n = 118), 5-10 years (n = 147), 11-15 years (n = 46), >15 years (n = 14). Various oral antidiabetic combinations used was obtained by reviewing the medical history chart. The therapy was then classified into sulphonyl ureas + metformin (n = 112), dpp-4 inhibitors + metformin (n = 151), α-glucosidase inhibitor + metformin (n = 48) and thiazolidinedione + metformin (n = 14).

Measurement of Covariates: Risk factors, other than diabetes, were evaluated to find its individual influence on cognitive functions. Demographic data such as age, sex, educational status, and smoking status (current smoker, smoker but quit, both smoker and alcohol user) were collected using a self-reported questionnaire. Presence of hypertension was ascertained by self-report or by use of antihypertensive medication.

Cognitive Function Assessment: A validated Tamil version of Folstein mini-mental state examination (MMSE) scale was used to assess the

cognitive function of the patients [16]. MMSE is a neuro-psychometric test containing 11-questions that measure various domains of cognitive function such as orientation, registration, attention and calculation, memory and language and visuospatial skills and has a maximum score of 30 points. The questionnaire was administered after making sure the participant is comfortable. The scores were categorized into three groups; severely-moderately impaired (0-17, n =15), mildly impaired (18-23, n = 63), and normal (24-30, n = 247).

Statistical Analyses: Statistical analysis was performed using the Graph Pad Instat 3 to find the association between different risk factors and oral antidiabetic drug with cognitive function. The association of different combination of oral antidiabetic drugs with individual domains of cognitive functions was also performed.

RESULTS: Baseline characteristics of the study participants are described in **Table 2**. A higher prevalence of type 2 diabetes mellitus was found in middle-aged patients.

We found that 85%of the participants had no social habits (n=265) and 4.3% of the participants had more than 15 years of diabetes (n=14).

TABLE 2: CHARACTERISTICS OF STUDY PARTICIPANTS AND ITS ASSOCIATED COGNITIVE FUNCTION (MEAN AND SD)

Variables (N = 325)		F	%	Average of MMSE scores
Age	25-35	12	3.69	29.67
	36-45	63	19.38	29.41 ± 0.18
	46-55	141	43.38	27.65 ±0.32
	56-65	60	18.46	24.95 ±0.52
	66-75	38	11.69	22.68 ±0.62
	>75	11	3.38	18.18 ±1.34
Gender	Female	137	42.15	26.01 ± 0.40
	Male	188	57.84	26.68 ± 0.24
Education	Up to 5 years	97	29.85	22.22 ± 0.52
	6 – 12 years	101	31.08	26.85 ± 0.37
	≥15 years	127	39.07	28.92± 0.16
Social Habits	Smokers	12	3.69	29.05 ± 0.42
	Smokers who quit	26	8	27.12 ± 0.69
	Alcohol users	16	4.92	25.75 ± 1.35
	Both smoking and alcohol user	6	1.85	28.83 ± 0.75
	None	265	81.54	26.51 ± 0.27
Duration of disease	6months - 5 years	118	36.08	27.72 ± 0.37
	5-10 years	147	45.23	26.56 ± 0.35
	11-15 years	46	14.15	25.98 ± 0.62
	>15 years	14	4.3	21.36 ± 1.37
Co-morbid conditions	Hypertension	75	23.08	24.27 ± 0.49
	None	250	76.92	27.4 ± 0.26

Metformin was the most commonly prescribed drug, in combination with other oral antidiabetics. Dpp- IV+ metformin (n= 151) and sulphonylureas + metformin (n= 112) were the most commonly prescribed oral antidiabetic combinations making up for more than 80% of the prescriptions.

TABLE 3: DIFFERENT COMBINATIONS OF ANTIDIABETIC DRUGS AND THE MEAN MMSE SCORES (MEAN + SD)

Drug Class	F	%	Mini-mental state examination
			Mean ± SEM
Group A: Metformin-sulphonylureas	112	34.46	24.64 ± 0.38(*)
Group B: Metformin-DPP-IV inhibitors	151	46.46	29.11 ± 0.19(**) (***)
Group C: Metformin-Alpha glucosidase Inhibitors	48	14.77	25.33 ± 0.73
Group D: Metformin-thiazolidinediones	14	4.31	21.36 ± 1.77

*p<0.001 for (Group A *vs*. Group B) **p<0.01 for (Group B *vs*. Group C) ***p<0.05 for (Group B *vs.* Group D)

Notably, 76% of the type 2 diabetes mellitus patients had no cognitive impairment (n=247). There was a slight difference between the mean MMSE scores of male and female patients (26.01 ± 0.40, 26.68 ± 0.24) respectively. A trend to decrease in MMSE scores with aging was found,

with a few participants of 25-35 years maximum score of 29.67. Patients whose age was above 75 years, had a mean score of 18.18 ± 1.34. Those who had no specific social habits had a mean MMSE score of 26.51 ± 0.27.

The significant difference of MMSE scores was among diabetes patients with hypertension (24.27 ± 0.49) compared to diabetes only patients (27.4 ± 0.26). An increase in the duration of diabetes shows a trend in the decrease in mean MMSE scores ranging from 27.72 ± 0.37 to 21.36 ± 1.37.

Table 3 presents the comparisons of different combinations of oral antidiabetic drugs and their mean MMSE scores. We categorized the participants into four groups; Groups A (metformin - sulphonylureas), B (metformin-dpp-iv inhibitors), C (metformin-alpha glucosidase inhibitors), and D (metformin-thiazolidinediones) and assessed their mean MMSE scores. Group B had significantly higher MMSE scores compared to both group C and group D (P<0.001). We also found a significant reduction in mean MMSE scores for Group A compared to Group B (p<0.01).

TABLE 4: ASSOCIATION BETWEEN DIFFERENT TYPES OF ORAL ANTIDIABETIC DRUGS AND COGNITIVE FUNCTIONS OF INDIVIDUAL DOMAIN

Drug class	Orientation	Registration	Attention	Memory	Language & Visio-spatial skills
	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
Group A: Sulphonylureas + Metformin	9.03 ± 0.11[1(B)]	2.68 ± 0.06[1(B)]	3.35 ± 0.21[1(B)]	2.37 ± 0.08[1(B)]	7.21 ± 0.15[1(B)]
Group B: DPP-IV Inhibitors + Metformin	9.93 ± 0.03[1(C)]	2.97 ± 0.01[1(C)]	4.48 ± 0.12[1(D),2(C)]	2.99 ± 0.29[1(C, D)]	8.73 ± 0.06[1(C, D)]
Group C: Alpha glucosidase inhibitor + Metformin	9.22 ± 0.18	2.78 ± 0.08	3.54 ± 0.31	2.44 ± 0.14	7.38 ± 0.23
Group D: Thiazolidinedione's + Metformin	9.14 ± 0.44	2.5 ± 0.3	1.79 ± 0.66	1.7 ± 0.29	6.2 ± 0.7

1 = P<0.001 for (Study group) *vs*. (Group excluding study group) 2 = P<0.01 for (Study group) vs. (Group excluding study group)

DISCUSSION: Glycaemic control has little influence in preventing T2DM from being the risk factor for dementia and is negatively correlated with glycosylated HB [15, 16]. In this regard, we opted to use the duration of disease rather than blood glucose. A decrease in mean cognitive scores with an increase in duration of disease was observed in this study. For instance, in a study conducted by Hassing *et al*., a longer duration of disease was associated with vascular problems, the pathology for vascular dementia [17, 18]. Hyperglycaemia has also been implicated in cases such as end-organ damage, hypoglycemia, brain insulin resistance, *etc*. [19]

In our study, smokers had higher cognitive functions, which are in contrast with previous studies. However, smoking has been associated with declines in verbal memory, visual search speeds, and information processing speed in patients with diabetes in other studies [20, 21]. We also did not find a significant difference in cognitive function between male and female patients. In contrast, one study found that females had a greater cognitive decline compared to men [21]. However, that might be only after the post-ovulation period, especially in the domain of attention, although, they had the upper hand in executive functions compared to male [22].

Patients who had a greater number of years of education were found to have better cognitive functions compared to those who had lesser number of years of education. For instance, cognitive reserve and brain plasticity are believed to be potentiated with the education that delays the clinical expression of cognitive impairment [23]. We found that patients who had 15 or a greater number of years of education had better MMSE score means than other groups. This could imply that education protects against cognitive impairment later in life.

Various animal and cell line studies have explored neuroprotective and cognitive enhancing effects of metformin, mainly by reduction of oxidative stress and insulin resistance [25-27, 11]. However, metformin might cause vitamin B12 deficiency, vital for cognition, and patients on metformin had poor cognitive performance compared to patients without metformin [10]. However, in our study, 95% of our participants were having multivitamin

prescribed by a physician, which alleviates the harmful effect of metformin on cognition (Data not showed).

Metformin is the first line drug in the treatment of diabetes and is common in all the groups selected for the study. Since it is the first line drug in the clinical setting, other antidiabetic medicines are usually taken in combination with metformin. In the previous study, patients on metformin had a better effect on cognitive functions [25]. In this study, metformin-sulphonylurea combination therapy had better cognitive functions compared to metformin-thiazolidinedione combination; however, the effect was lower than in metformin-alpha glucosidase inhibitors combination. The effect, however, lacks statistical significance may be due to a small sample size of this study. Also, metformin-DPP-IV inhibitors combination group had higher MMSE scores, irrespective of the cognitive domain, compared to metformin-sulphonylurea combination group. Sulphonylureas may induce hypoglycemia and fluctuations in blood glucose level compared to DPP-IV inhibitors. However, this study did not investigate the frequency of hypoglycaemic attacks during the duration of treatment. In contrast to our findings, Hsu *et al*., reports that metformin-sulphonylurea combination reduces the risk of dementia by 35% on administration for more than 8 years [15].

A significant effect was observed to participants who used DPP iv inhibitors + metformin in cognitive functions compared to all other oral antidiabetic combination treatment groups. Dpp iv reported to reduce the brain oxidative stress, restores hippocampal mitochondrial function, and neurogenesis prevents learning and memory impairment [13]. Sitagliptin improved memory by hippocampal neurogenesis [26]. Sitagliptin was found to aggravate the tau phosphorylation in insulin-resistant brains [27]. In the transgenic mice model, early administration of sitagliptin delays the amyloid deposition and some forms of AD pathology [28].

The present study has several limitations that we would like to acknowledge. For instance, because this is of its cross-sectional nature, the exposure-effect relationship cannot be confirmed, although it might hint an association. Furthermore, there are many factors which may confound the results including; age, blood sugar level, hypertension, other comorbid conditions, and use of non-antidiabetic medications which we could not control in the analysis. Also, we did not measure patient compliance with the medications and diet control measurement. A lack of brain imaging made it impossible to rule out other neurological diseases. Hence, these results must be interpreted with caution.

In conclusion, this study has strengths. First, the microanalysis of multiple cognitive domains (individual domain) was done and found a significant association with the type of oral diabetes medicines. Additionally, variables like age, duration of disease, education, *etc*., which have a direct influence on cognition were measured. Importantly, to the best of our knowledge, this is one of the primary studies which analyzed the effect of combination therapy on individual cognitive domains.

CONCLUSION: A combination of metformin and DPP IV inhibitors showed better cognitive scores in five cognitive domains using MMSE as a cognitive assessment tool. The association between the combination of anti-diabetes medication and cognitive function warrants further longitudinal studies.

ACKNOWLEDGEMENT: The authors acknowledge the Indian Council of Medical Research (ICMR) for providing financial assistance as fellowship during the process of this manuscript. The content is solely the responsibility of authors and does not represent the official views of ICMR.

CONFLICT OF INTEREST: Authors declared no conflicts of interest.

REFERENCES:

1. Kumar S, Misra A, Verma AK, Roy R, Tripathi A and Ansari KM: Diabetes in India: A long way to go. International Journal of Scientific Reports 2015; 1: 1-2.
2. Kumar R, Jeffrey CL and Raphael B: Type 2 diabetes mellitus, cognition and brain in aging: A brief review. Indian J Psychiatry 2009; 51: S35-S38.
3. Kataria L, Pandya H, Shah S, Shah H and Gerg R: Prevalence and pattern of cognitive dysfunction in type 2 diabetes mellitus. International Journal of Medical and Applied Sciences 2013; 2: 245-52.
4. Arvanitakis Z, Wilson RS, Li Y, Aggarwal NT and Bennett DA: Diabetes and function in different cognitive

systems in older individuals without dementia. Diabetes Care 2006; 29: 560-5.
5. Knopman DS and Roberts R: Vascular risk factors: Imaging and neuropathologic correlates. Journal of Alzheimer's Disease 2010; 20: 699-09.
6. Alagiakrishnan K, Sankaralingam S, Ghosh M, Mereu L and Senior P: Antidiabetic drugs and their potential role in treating mild cognitive impairment and alzheimer's disease. Discov Med 2013; 16: 277-86.
7. Hewer W, Mussell M, Rist F, Kulzer B and Bergis K: Short -term effects of improved glycaemic control on cognitive function in patients with type 2 diabetes. Gerontology 2003; 49: 86-92.
8. Mussell M, Hewer W, Kulzer B, Bergis K and Rist F: Effects of improved glycaemic control maintained for 3 months on cognitive function in patients with type 2 diabetes. Diabet Med 2004; 21: 1253-6.
9. Imfeld P, Bodmer M, Jick SS and Meier CR: Metformin, other antidiabetic drugs, and risk of Alzheimer's disease: A population-based case-control study. J Am Geriatr Soc 2012; 60: 916-21.
10. Moore EM, Mander AG, Ames D, Kotowicz MA, Carne RP and Brodaty H: Increased risk of cognitive impairment in patients with diabetes is associated with metformin. Diabetes Care 2013; 36: 2981-7.
11. Koo IH, Kim Y, Jung EJ, Shin JH, Choi JW and Won MH: Metformin normalizes type 2 diabetes-induced decrease in cell proliferation and neuroblast differentiation in the rat dentate gyrus. Neurochem Res 2010; 35: 645-50.
12. Watson GS, Cholerton BA, Reger MA, Baker LD, Plymate SR and Asthana S: Preserved cognition in patients with early Alzheimer disease and amnestic mild cognitive impairment during treatment with rosiglitazone: A preliminary study. Am J Geriatr Psychiatry 2005; 13: 950-8.
13. Pintana H, Apaijai N, Chattipakorn N and Chattipakorn S: DPP-4 inhibitors improve cognition and mitochondrial brain function of insulin-resistant rats. Journal of Endocrinology 2013; 218: 1-11.
14. Farlow MR: Treatment of mild cognitive impairment (MCI). Current Alzheimer Research 2009; 6: 362-7.
15. Hsu C, Wahlqvist ML, Lee MS and Tsai HN: Incidence of dementia is increased in type 2 diabetes and reduced by the use of sulfonylureas and metformin. Journal of Alzheimers Disease 2011; 24: 485-93.
16. Roy S, Kim N, Desai A, Komaragiri M, Baxi N and Jassil N: Cognitive function and control of type 2 diabetes mellitus in young adults. North American Journal of Medical Sciences 2015; 7: 6-13.
17. Hassing LB, Johansson B, Pedersen NL, Sven EN, Berg S and McClearn G: Type 2 diabetes mellitus and cognitive performance in a population-based sample of the oldest old : Impact of comorbid dementia. Aging, Neuropsychology, and Cognition 2003; 10: 99-07.
18. Roberts OR, Geda YE, Knopman DS, Christianson TJH, Pankratz VS and Boeve BF: Association of duration and severity of diabetes mellitus with mild cognitive impairment. Arch Neurol 2008; 65: 1066-73.
19. Christopher TK and Elizabeth RS: Cognitive Dysfunction and Diabetes Mellitus. Endocrine Reviews 2008; 29: 494-11.
20. Ruis C, van den Donk M, Biessels GJ, Kapelle LJ, Gorter K and Rutten GEHM: Cognition in the early stage of type 2 diabetes. Diabetes Care 2009; 32: 1261-65.
21. EW Gregg, Yaffe K, Cauley JA, Rolka DB, Blackwell TL and Narayan KMV: Is diabetes associated with cognitive impairment and cognitive decline among older women? Arch Intern Med 2000; 160: 174-80.
22. Upadhyay N and Guragain S: Comparison of cognitive functions between male and female medical students: A pilot study. J Clin Diagnostic Res 2014; 8: 12-5.
23. Carret NL, Lafont S, Letenneur L, Dartigues JF, Mayo W and Fabrigoule G: The effect of education on cognitive performances and its implication for the constitution of the cognitive reserve. Developmental Neuropsychology 2003; 23: 317-37.
24. El-Mir MY, Detaille D, Villanueva GR, Delgado-Esteban M, Guigas B and Attia S: Neuroprotective role of antidiabetic drug metformin against apoptotic cell death in primary cortical neurons. J Mol Neurosci 2008; 34: 77-87
25. Lin Y, Wang K, Ma C, Wang X, Gong Z and Zhang R: Evaluation of metformin on cognitive improvement in patients with non-dementia vascular cognitive impairment and abnormal glucose metabolism. Frontiers in Aging Neuroscience 2018; 10: 227.
26. Gault VA, Lennox R and Flatt PR: Sitagliptin, a DPP-4 inhibitor, improves recognition memory, oxidative stress, hippocampal neurogenesis and up-regulates key genes involved in cognitive decline. Diabetes Obes Metab 2015; 17: 403-13.
27. Kim DH, Huh JW, Jang M, Suh JH, Kim TW and Park JS: Sitagliptin increases tau phosphorylation in the hippocampus of rats with type 2 diabetes and in primary neuron cultures. Neurobiology of Disease 2012; 46: 52-8.
28. Amico MD, Filipo DC, Marfella R, Abbatecola AM, Ferraraccio F and Rossi F: Long-term inhibition of dipeptidyl peptidase-4 in alzheimer's prone mice. Exp Gerontol 2010; 45: 202-7.

How to cite this article:
Nair NK, Vidhya N, Mingate MD and Vijayakumar PRA: Comparison of different classes of oral antidiabetic drugs in combination with metformin on cognitive functions. Int J Pharm Sci & Res 2019; 10(7): 3455-60. doi: 10.13040/IJPSR.0975-8232.10(7).3455-60.