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Efficacy of addition of the anti-inflammatory, IV glutathione to standard ketamine IV therapy in major depressive disorder

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ABSTRACT

Ketamine, a N-methyl-D-aspartate (NMDA) antagonist, is used for treatment-resistant depression (TRD). Recent studies have shown that there are increased levels of pro-inflammatory cytokines in individuals with major depressive disorder (MDD) and those with higher levels of oxidative stress markers have a decreased or null response to conventional antidepressants. Glutathione (GSH) as an antioxidant adjuvant to ketamine has not been well studied. This double-blind study with 30 patients divided into 2 groups of 15 each, aimed to determine if GSH, added to standard ketamine infusion (GSH+K), rendered better outcomes in MDD patients versus patients receiving ketamine infusions with a normal saline placebo (K+NS). There were significant drops in BDI-II scores from day 1 to day 14, PHQ- scores from day 1 to day 14 and PHQ-9 scores day 14 to day 28, suggesting the overall treatment was effective. There were no statistically significant differences between the groups over time. However, a sustained improvement in depressive symptoms was observed for 14 days post-infusion in both groups.

1. Introduction

Depression affects more than 16 million adults in the United States, with health care costs and productivity losses amounting to $210 billion annually (Quantifying the cost of depression). Major depressive disorder (MDD) is a common form of depression, and is diagnosed when symptoms of depression last for longer than two weeks at a time and interfere with one's ability to perform activities of daily living (Lindqvist et al., 2017, Dowlati et al., 2010). MDD has a lifetime prevalence of 15–20 % in the general population, during which time an individual is at risk of experiencing a major depressive episode (MDE) (Lindqvist et al., 2017, Dowlati et al., 2010). Likewise, individuals with MDD may experience a decreased quality of life, feelings of worthlessness and hopelessness, a decreased ability to perform at work or in school, weight loss or gain, suicidal ideations, or even suicide attempts (Dowlati et al., 2010).

Traditional antidepressant pharmacotherapy is based on the theory that depression results from a dysfunction in monoamine activity within the central nervous system (CNS) (Lindqvist et al., 2017, Dowlati et al., 2010, Hollon and Shelton, 2001). First-line antidepressant agents, such as selective serotonin reuptake inhibitors (SSRIs) and selective norepinephrine reuptake inhibitors (SNRIs), modulate the neurotransmitters serotonin and norepinephrine, respectively, to improve symptoms of depression; however, approximately one-third of individuals do not experience any relief (Lindqvist et al., 2017, Dowlati et al., 2010, Voineskos et al., 2020). Individuals with treatment-resistant depression (TRD) do not experience relief of depression symptoms despite conventional treatments and are at a greater risk for suicide. Suicide is the tenth leading cause of death in the U.S. and the second leading cause of death for individuals 10–34 years of age (Suicide 2021).

A novel approach for TRD is aimed at enhancing neuroplasticity rather than targeting the monoamines themselves (Harmer et al., 2017). Most notably, ketamine, an NMDA receptor antagonist, works via this

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mechanism (Voineskos et al., 2020, Zanos and Gould, 2018). Ketamine is a racemic mixture of R-ketamine and S-ketamine enantiomers, with S-ketamine having a higher affinity for the NMDA receptor, originally alluded to for its more potent antidepressant effect (Thase and Connolly, 2020, Singh et al., 2015). However, new evidence suggests that the downstream effects of NMDA receptor antagonism and/or a ketamine metabolite includes activation of α-amino-3--hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptor, which promotes synaptic plasticity, as well as increased glutamate cycling in the prefrontal cortex (Thase and Connolly, 2020, Harmer et al., 2017, Zanos and Gould, 2018). After a single subanesthetic infusion, this drug provides antidepressant effects within hours, lasting for about a week (Thase and Connolly, 2020, Zanos and Gould, 2018).

Nonetheless, other biological influences, separate from a neurotransmitter deficit, are now being researched as causes of MDD (Lindqvist et al., 2017, Dowlati et al., 2010, Voineskos et al., 2020). Inflammation, oxidative stress, and antioxidant reduction have been studied in recent years and are being cited as co-contributors to the pathogenesis of MDD (Lindqvist et al., 2017, Dowlati et al., 2010, Voineskos et al., 2020). Studies have also shown that increased levels of pro-inflammatory cytokines and oxidative stress markers are found in individuals with MDD and that those with higher levels of oxidative stress markers have a decreased or null response to conventional antidepressant treatments (Lindqvist et al., 2017, Dowlati et al., 2010, Czarny et al., 2018).

Studies have shown that glutathione (GSH) levels of individuals with MDD are significantly lower compared to their healthy counterparts (Freed et al., 2017). Glutathione, a major intracellular antioxidant, works to maintain redox homeostasis in the body by neutralizing reactive oxygen species (ROS) (Cobley et al., 2018). Several lines of evidence suggest that ROS concentrations are elevated during inflammation and enhance tissue damage through activation of pro-inflammatory cytokines (Silvagno et al., 2020, Rose et al., 2012). These cytokines, such as TNF alpha, in turn, generate additional ROS, perpetuating a positive feedback loop that amplifies the inflammatory state (Hao et al., 2022, Hoffmann and Griffiths, 2018). GSH concentrations have been shown to influence the level of inflammation in several models of disease, Rose et al. (2012), Hao et al. (2022). This may reflect, in part, the capability of GSH to reduce ROS levels. Glutathione is the most abundant antioxidant in the brain with an affinity for glucose and oxygen and assumes primary responsibility over the preservation of existing synaptic connections and neuronal networks (Gawryluk et al., 2011, Gibson et al., 2012). By protecting the existing neuronal circuitry, GSH may enhance ketamine's known mechanism of action. One study measured in vivo cortical GSH concentrations in adolescents with MDD ($n = 19$) versus healthy controls ($n = 8$) and found that adolescents with MDD had lower somatic levels of GSH compared to their healthy counterparts (CI = 95 %) (Freed et al., 2017). This suggests that decreased GSH levels within the brain may contribute to the pathogenesis of psychological illness, such as MDD (Freed et al., 2017, Gawryluk et al., 2011, Gibson et al., 2012).

Currently, there is a gap in the literature regarding the use of intravenous (IV) GSH as an adjunct treatment for depression. There has been increasing interest in using N-acetylcysteine (NAC), the precursor to glutathione, as an adjunctive treatment for depression. NAC has antioxidant, anti-inflammatory, and neuroprotective properties (Zheng et al., 2018, Berk et al., 2008, Kishi et al., 2020). When given as an oral supplement, it appears that NAC may have significant effects on depression scores in studies lasting longer than sixteen weeks (Berk et al., 2008; Kishi et al., 2020; Berk et al., 2014). Given the significant association between treatment with NAC and decreased depressive symptoms, treatment with glutathione has potential benefits as an adjunctive treatment for MDD.

Therefore, the overarching hypothesis of the project was that individuals with reduced baseline GSH levels and/or higher levels of oxidative stress, such as those with TRD, may benefit from supplemental GSH.

The purpose of this study was to determine if the addition of a glutathione infusion together with a standard ketamine infusion (GSH+K) led to better outcomes in mood disorder patients compared to a standard ketamine infusion plus normal saline placebo (K+NS), as measured by Patient Health Questionnaire 9-item (PHQ-9) and Beck Depression Inventory-II (BDI-II) scores in subjects with MDD.

2. Materials and methods

2.1. Study design

This was a randomized, controlled, and double-blind study ($n = 30$) whereby after IRB approval, a total of 30 subjects with a clinical diagnosis of MDD per DSM-5 were enrolled in the study. The patients were deemed to be treatment resistant (TRD) defined by the study psychiatrists after failing to respond to two different classes of antidepressant medications. Subjects from two outpatient ketamine and pain management clinics were randomly assigned to control and experimental groups.

A total of 38 patients were screened. Eight patients were excluded based on exclusion criteria. Exclusion criteria included patients deemed to have multiple mental health diagnoses ($N = 3$), and those that did not meet the definition of TRD for the study (N=5). Randomization was employed by use of computer generated assigments.

This study's specific aim was to analyze de-identified data obtained from a randomized, controlled, and double-blind study ($n = 30$). The purpose of this study was to determine if the addition of a glutathione infusion together with a standard ketamine infusion (GSH+K) led to better outcomes in mood disorder patients compared to a standard ketamine infusion plus normal saline placebo (K+NS), as measured by Patient Health Questionnaire 9-item (PHQ-9) and Beck Depression Inventory-II (BDI-II) scores in subjects with MDD.

A minimum sample size of 18 subjects was calculated utilizing the G*Power 3.1 software system considering an $\alpha = 0.05$, test power 80 %, and an effect size, d, of 1.3. An effect size of 1.3 was decided upon based on the reported effect sizes from a study that utilized the PHQ-9 scale as the primary measurement tool (Fava et al., 2020). After IRB approval, a total of 30 subjects with a clinical diagnosis of MDD were enrolled in the study. Subjects were randomly assigned to control and experimental groups, with 15 subjects in each group. All subjects, regardless of group assignment, received six treatments of IV ketamine over a period of 2 weeks, with doses ranging from 0.5–1.0 mg/kg/hr infusing over 50–60 min. Specific dosing was determined by patient response and standardized clinic protocols. The control group received a placebo infusion of 50mL of NS over 15 min after the ketamine infusion. Subjects in the experimental group received 2,000 mg of glutathione mixed in 50mL of NS over 15 min after the ketamine infusion. Subjects completed PHQ-9 and BDI-II depression scales prior to infusion therapy (day 0), at two weeks (day 14), and at four weeks (day 28) post-infusion to assess for any added anti-inflammatory effects on efficacy. Data was compiled and stripped of all patient identifiers for data analysis by the research team.

After completion of the randomized, controlled, and double-blind study, the research team was provided with a password-protected and encrypted Excel spreadsheet containing study results for statistical analysis. Data contained in the spreadsheet was stripped of all unique patient identifiers. This data was compiled and de-identified by an unblinded research team member at the clinic. The research team conducting the data analysis did not have access to patient medical records or involvement with patient interactions or treatments conducted at the clinic.

The Excel spreadsheet contained de-identified demographics such as age and gender, ketamine dosing, treatment received (GSH+K or NS+K), and results of the PHQ-9 and BDI-II surveys administered prior to infusion therapy (day 0), at two weeks (day 14), and at four weeks (day 28) post-infusion. The data analysis compared the efficacy of K+NS versus K+GSH adjunct in subjects with MDD. The data analysis focuses

on the results of both de-identified PHQ-9 and BDI-II surveys as outcome measures.

2.2. Measurement of PHQ-9 and BDI-II

The PHQ-9 was the primary outcome measure. It is a self-administered, 9-item questionnaire where the individual ranks depressive symptoms from 0 (not at all) to 3 (nearly every day) over the last two weeks (Manea et al., 2012, He et al., 2020). The summed-item score ranges between 0–27 to assess depression severity, with a score greater than or equal to 10 being the cut off for diagnosing MDD (Manea et al., 2012; He et al., 2020; Kroenke et al., 2001). The reliability and validity of the PHQ-9 has been well established with test-retest reliability of 0.84 and sensitivity and specificity consistently being reported between 0.85–0.88 (He et al., 2020, Kroenke et al., 2001, Manea et al., 2015). A change in PHQ-9 score of 5 or greater reflects clinical improvement in those being treated for depression (Löwe et al., 2004).

The secondary outcome measure, the BDI-II, is a self-administered, 21-item questionnaire, where an individual self-reports a number from 0–3 that most accurately reflects depressive attitudes and symptoms over the last two weeks (Stockings et al., 2015). Analysis of the BDI-II is performed by calculating the sum of each item scored, which ranges between 0–63 (Manea et al., 2012, He et al., 2020, Kroenke et al., 2001). Scores ranging 0–13 reflect minimal depression, 14–19 mild depression, 20–28 moderate depression, and 29–63 reflects severe depression (Stockings et al., 2015, Smarr and Keefer, 2011, Furukawa, 2010). No clear cutoff score for MDD has been identified. However, studies suggest cutoff values of greater than or equal to 16 and 18 in varying patient populations (Smarr and Keefer, 2011, Arnau et al., 2001, Huffman et al., 2010, Hiroe et al., 2005). Reliability and validity of the BDI-II have been established in multiple studies with a reliability of 0.86–0.94 and sensitivity and specificity of 0.88–0.94 being reported (Stockings et al., 2015, Arnau et al., 2001, Huffman et al., 2010, Hiroe et al., 2005). A decrease in BDI-II score of 5 points is the minimal change in score to reflect clinical improvement, a change in score of 10–19 reflects a moderate improvement, and a change greater than or equal to 20 points reflects a large improvement (Hiroe et al., 2005).

2.3. Statistical analysis

To assess whether statistically significant changes occurred in PHQ-9 and BDI-II, Repeated-Measures ANOVA was used to analyze the scores of the GSH+K experimental group to the NS+K control group for both PHQ-9 and BDI-II scores. The assessed intervals included from pre-intervention (day 0) to 14 days post-intervention and from pre-intervention (day 0) to 28 days post-intervention. Repeated-Measures ANOVA was used to compare mean test scores repeated three times, before the intervention and twice after the intervention, while simultaneously testing differences between experimental and control groups. If there were significant differences over time and by group in the ANOVA results, independent samples *t*-tests using Bonferroni adjustments were used as post hoc analyses to compare a mean change in scores of the GSH+K experimental group to the NS+K control group for both PHQ-9 and BDI-II scores from pre-intervention (day 0) to 14 days post-intervention and from pre-intervention (day 0) to 28 days post-intervention. All tests used an alpha of .05 (CI of 95 %) to determine statistical significance and ran using SPSS 29.

3. Results

The data set consisted of de-identified data from 30 individuals with MDD. In total, 17 participants were female, and 13 participants were male. The gender distribution of the control and treatment groups was compared using a Chi-square test. Age, weight, ketamine dosing, BDI-II, and PHQ-9 between the two groups were compared using independent samples *t*-tests. Table 1 displays an analysis of the data set ($n = 30$) that revealed no statistically significant differences in gender ($p = 0.71$), age ($p = 0.83$), or weight ($p = 0.67$) at baseline (day 0) between subjects in both the experimental (GSH+K) and control (NS+K) groups. Moreover, the experimental and control groups did not significantly differ in ketamine dose ($p = 0.21$), BDI-II scores ($p = 0.07$), or PHQ-9 ($p = 0.06$) scores at baseline.

Table 1
Participant characteristics by group

	Experimental ($n = 15$)	Control ($n = 15$)	*P*
Male, *n (%)*	7 (46.7)	6 (40.0)	.71
Age in years, *M* (*SD*)	39.0 (9.7)	38.2 (10.7)	.83
Weight in kg, *M* (*SD*)	80.3 (16.9)	77.2 (22.0)	.67
K Dose mg/kg, *M* (*SD*)	4.03 (0.31)	3.88 (0.30)	.21
BDI pre, *M* (*SD*)	31.1 (12.7)	38.6 (8.5)	.07
PHQ pre, *M* (*SD*)	14.8 (5.2)	18.3 (4.3)	.06

Note: BDI-Beck Depression Inventory, PHQ-Patient Health Questionnaire, K-Ketamine

To examine whether changes in BDI-II or PHQ-9 varied by gender, repeated measures ANOVAs were run with gender as a factor. There were no differences in BDI-II or PHQ-9 over time by gender. Repeated Measures ANOVAs with age and weight as covariates also showed no differences in BDI-II or PHQ-9 by age or weight.

Mean baseline PHQ-9 scores on day 0 were 14.8 and 18.3 for the experimental (GSH+K) and control (NS+K) groups, respectively. Mean scores on day 14 decreased to 8.3 for the experimental group and 9.5 for the control group. Furthermore, mean scores on day 28 were 7.3 for the experimental group and 7.6 for the control group. The drop in mean PHQ-9 scores for both groups was statistically significant from day 0 to day 14 ($p < 0.001$) and from day 14 to day 28 ($p = 0.02$). Table 2 and Fig. 2 both show that there was no statistically significant difference between the two groups ($p = 0.15$).

Mean baseline BDI-II scores on day 0 were 31.1 and 38.6 for the experimental (GSH+K) and control (NS+K) groups, respectively. Mean scores on day 14 decreased to 18.1 for the experimental group and 21.5 for the control group. Furthermore, mean scores on day 28 decreased to 15.7 for the experimental group and 18.5 for the control group. Although the scores dropped significantly from day 14 to day 28, this was not statistically significant ($p = .12$). However, the drop in BDI-II scores for both groups from day 0 to day 14 was statistically significant ($p < 0.001$). As shown in Table 2 and Fig. 1, there was no statistically significant difference in mean BDI-II scores between the experimental and control groups over time ($p = 0.49$).

Table 2 and Figs. 1 and 2 compare BDI-II and PHQ-9 from day 0 to day 14 to day 28 by treatment. Repeated measures ANOVAs were used to compare BDI-II and PHQ-9 over time with treatment conditions as a

Table 2
Comparison of BDI and PHQ Over time by treatment condition

	Experimental	Control	*Repeat Measures ANOVA*		
	($n = 15$)	($n = 15$)	*F*(2, 27)	*P*	η^2
BDI, Full Model			41.9	< .001	.76
Day 0, *M* (*SD*)	31.1 (12.7)	38.6 (8.5)			
Day 14, *M* (*SD*)	18.1 (13.8)	21.5 (15.0)	63.3	< .001	.69
Day 28, *M* (*SD*)	15.7 (14.3)	18.5 (14.3)	2.6	.12	.09
BDI * Treatment			0.7	.49	.05
PHQ, Full Model			67.9	< .001	.83
Day 0, *M* (*SD*)	14.8 (5.2)	18.3 (4.3)			
Day 14, *M* (*SD*)	8.3 (6.8)	9.5 (6.4)	83.4	< .001	.75
Day 28, *M* (*SD*)	7.3 (6.7)	7.6 (5.1)	6.1	.02	.06
PHQ * Treatment			2.0	.15	.13

Note: ANOVA = Analysis of variance; η^2= eta squared, effect size for ANOVA. F, P, and η^2 based on multivariate tests for Full Model and interaction between time (day 0, 14 and 28 respectively) and experimental group. F, P, and η^2 for individual times based on within-subject contrast with the previous time.

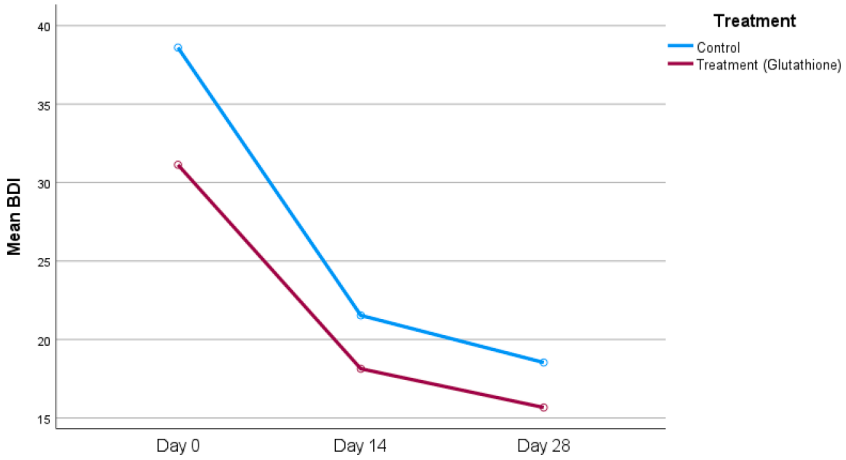

Fig. 1. Mean BDI-II over time by treatment condition.

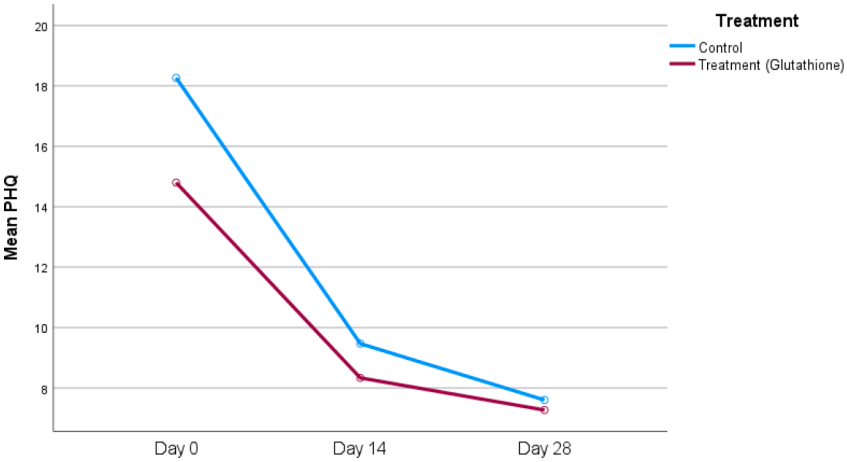

Fig. 2. Mean PHQ-9 over time by treatment condition.

factor. Although day 14 and day 28 measures were slightly skewed, ANOVA is robust enough to slight deviations in normality. Therefore, ANOVA was still deemed appropriate.

4. Discussion

This study aimed to evaluate the effectiveness of IV GSH as an adjuvant to IV Ketamine treatment in improving mood scores in subjects with MDD, and to determine if the treatment effects were long-lasting at four weeks. To date, the use of IV GSH to treat MDD has not been reported in the literature. Moreover, IV GSH as an adjunct to IV Ketamine in MDD has not been closely studied. Nonetheless, IV GSH has been safely administered to individuals with Parkinson's Disease (PD), and for the prevention of contrast medium-induced nephropathy with positive results and a lack of serious adverse effects (Sechi et al., 1996, Hauser et al., 2009, Saitoh et al., 2011). The safety and efficacy of oral GSH supplementation has also been documented in 2 RCTs which reported an absence of adverse reactions (Richie et al., 2015, Allen and Bradley, 2011).

Miller et al. (2009) extrapolate on how individuals with major depression demonstrate inflammation via elevated levels of inflammatory cytokines in peripheral blood as well as in central circulation via cerebral spinal fluid (CSF). (Miller et al., 2009) Moreover, treatment implications of the inflammation-depression hypothesis suggest that treatments should target the immune system and its impact on the brain (Miller et al., 2009). Furthermore, Miller and Raison (2016) continue to research the relationship between inflammation and MDD and the resistance that may come with current antidepressant therapies. (Miller and Raison, 2016) Nearly a decade later, the authors suggest that individuals who do not display elevated levels of peripheral inflammatory markers may not necessarily benefit from anti-inflammatory treatments (Miller and Raison, 2016).

However, GSH levels have been found to be depleted in individuals with MDD, suggesting this population may have a reduced capacity to neutralize reactive oxygen species (ROS) (Gawryluk et al., 2011). This is problematic because ROS directly damage tissues and promote the expression of pro-inflammatory cytokines (Lindqvist et al., 2017, Silvagno et al., 2020, Hao et al., 2022, Hoffmann and Griffiths, 2018). Therefore, it was hypothesized that individuals with reduced baseline GSH levels and/or higher levels of oxidative stress, such as those with TRD, may benefit from supplemental GSH. However, BDI-II and PHQ-9 score changes were not statistically significant in the experimental (GSH+K) group when compared to the control (NS+K) groups over time (PHQ-9*Treatment $P = 0.15$; BDI-II*Treatment $P = 0.49$). This suggests that the addition of GSH with ketamine may not provide an additional benefit. In both the experimental (GSH+K) and control (NS+K) groups, there were significant differences over time in both BDI-II and PHQ-9 scores. However, there were no statistically significant differences between the experimental (GSH+K) and control (NS+K) groups over time (see interaction terms in Table 2). The BDI-II and PHQ-9 scores showed significant decreases from day 0 to day 14, as measured by within-subject contrasts. Although BDI-II and PHQ-9 scores dropped

from day 14 to day 28, the difference was only statistically significant for PHQ-9 ($p = 0.02$), perhaps demonstrating a more sensitive test for mood in this population setting.

Upon further analysis, in both the experimental (GSH+K) and control (NS+K) groups, the mean change in score over time for both the PHQ-9 and the BDI-II reflect sustained clinical improvement in mood scores from day 0 to day 14 and from day 0 to day 28. A decrease in PHQ-9 scores greater than or equal to 5 reflects clinical improvement in depressive symptoms (Löwe et al., 2004). The mean decrease in PHQ-9 scores of the experimental (GSH+K) and control (NS+K) groups from day 0 to day 14 were 6.5 and 8.8, respectively. The mean decrease in PHQ-9 scores of the experimental (GSH+K) and control (NS+K) groups from day 0 to day 28 were 7.5 and 10.7, respectively. This suggests a clinical improvement from day 0 to day 14 and a continued and sustained improvement in depressive symptoms for 14 days post-infusion, as evidenced by a mean decrease in scores greater than 5 for both groups at all time points.

A decrease in BDI-II scores of 5 points is the minimal change in score to reflect clinical improvement, a change in score of 10-19 reflects a moderate improvement, and a change greater than or equal to 20 points reflects a large improvement (Hiroe et al., 2005). The mean decrease in BDI-II scores of the experimental (GSH+K) and control (NS+K) groups from day 0 to day 14 were 13 and 17.1, respectively. The mean decrease in BDI-II scores of the experimental (GSH+K) and control (NS+K) groups from day 0 to day 28 were 15.4 and 20.1, respectively. This suggests not only a moderate improvement in depressive symptoms from day 0 to day 14, but a moderate to large improvement in symptoms for 14 days post-infusion as evidenced by a mean decrease in score of 10-19 or greater than equal to 20 for both groups at all time points.

Repeated ketamine infusions have been shown to achieve a durable response period in depressive symptoms. A study performed by Murrough et al. (2013) assessed the long-term effects of repeated ketamine infusions over a period of 2 weeks and reported rapid reductions in mood scores that were sustained post-infusion with a median relapse of symptoms observed on day 18 (Murrough et al., 2013). Albot et al. (2018) examined the efficacy and durability of 6 ketamine infusions over a 12 day period in patients with MDD and reported a rapid reduction in depressive symptoms and a median relapse time of 20 days (Albott et al., 2018). In a similar study, Shiroma et al. (2014) reported that 45 % of those who were responsive to repeat ketamine infusions had not experienced a relapse of depressive symptoms at least four weeks post-infusion when ketamine infusions were given as an adjunct to oral treatments. (Shiroma et al., 2014) Moreover, the data of this study further support current research that the use of ketamine may rapidly improve mood scores with the use of repeated infusions and may continue to improve symptoms over two weeks even after infusions have stopped (Thase and Connolly, 2020, Singh et al., 2015, Murrough et al., 2013, Albott et al., 2018, Shiroma et al., 2014).

Ketamine's pharmacodynamic profile is favorable in patients who have TRD since effects are seen more instantaneously compared to conventional antidepressant therapies, such as selective serotonin reuptake inhibitors (SSRIs) (Thase and Connolly, 2020, Fava et al., 2020). Several randomized-control studies and systematic reviews have compared the efficacy and safety of ketamine in patients with TRD as single infusions and repeated infusions over time. Collective data from these studies suggest that improvement in depression scores is seen when at least a 0.5 mg/kg dose of Ketamine is administered (Singh et al., 2015, Xu et al., 2016, Fava et al., 2020, Murrough et al., 2013). After a single infusion, improvement in depression severity, as evidenced by improved depression scores of various scales, can be observed within hours, and can last for up to a week (Singh et al., 2015, Xu et al., 2016, Fava et al., 2020, Zanos and Gould, 2018). However, this study suggests that ketamine's effects could be sustained beyond one week which can have remarkable implications for individuals with MDD.

4.1. Strengths and Limitations

The findings of this data analysis are subject to some limitations. The primary limitation is the generalization of results to a larger population. The data analysis was conducted on a small sample size (N=30). Future studies where results are obtained from a larger geographical scope and sample size followed over a more extended time period may be better generalized to the public. Furthermore, potential social desirability bias can occur with PHQ-9 and BDI-II surveys. Despite the inevitable biases that can occur with these tools, these surveys have been repeatedly validated in their ability to diagnose depression and monitor response to treatments (He et al., 2020, Kroenke et al., 2001, Manea et al., 2015, Stockings et al., 2015, Arnau et al., 2001, Huffman et al., 2010, Hiroe et al., 2005). Additionally, future research should measure and compare the levels of inflammatory biomarkers in patients who are diagnosed with MDD to better cater treatment and conduct data analysis.

This study was conducted in a double-blind, randomized manner. One strength of this study is that the experimental (GSH+K) and control (NS+K) groups were comparable by all parameters, including age, weight, gender, drug dose, and baseline PHQ-9 and BDI-II scores. Thus, any differences in reported PHQ-9 and BDI-II scores can be related to the treatments rather than confounding factors. Additionally, all study participants were otherwise healthy without untreated medical conditions and diagnosed with MDD and TRD by a licensed psychiatrist. All of the PHQ-9 and BDI-II scores were collected in a standardized manner by a trained team. Another key strength of this study was the time frame allocated for patient response. Patients were required to complete response surveys on days 0, 14, and 28 of this experiment. The set dates allowed for 14-day periods of self-reflection. This time frame was crucial because it was long enough to allow for ample, recognizable cognitive changes yet short enough to promote accurate self-reflection.

5. Conclusion

This analysis of a randomized, double-blinded study examined and compared the mood outcomes of patients undergoing IV ketamine treatment for MDD with those undergoing IV ketamine and glutathione adjunct treatment for MDD. Results revealed significant drops in BDI-II scores from day 1 to day 14 and that the reductions from day 14 to 28 were not statistically significant, suggesting the effects of ketamine may last well beyond what others have proposed (Singh et al., 2015, Xu et al., 2016, Fava et al., 2020, Zanos and Gould, 2018). There was no significant difference between the experimental and control groups. Both groups had similar drops in BDI-II over time. There were also significant drops in PHQ-9 scores from day 1 to day 14 and from day 14 to 28. There was no difference between the experimental and control groups. Both groups had similar drops in PHQ-9 over time.

Study results yielded significant drops in BDI-II scores from day 1 to day 14, PHQ-9 scores from day 1 to day 14, and PHQ-9 scores day 14 to day 28, suggesting the overall treatment was effective. However, the study results yielded no statistical differences between the control and experimental groups in the BDI-II and PHQ-9 scores. This suggests that glutathione as an adjunct treatment to ketamine in TRD may not improve mood scores, though there may still be positive anti-inflammatory effects not reflected herein. Furthermore, this study suggests that previous studies may have underestimated the duration of efficacy in ketamine infusions for individuals with MDD.

Author statements

The authors of the original article titled *Efficacy of addition of the anti-inflammatory, IV Glutathione to standard Ketamine IV therapy in Major Depressive Disorder* attest that we have materially participated in the research, and/or manuscript preparation.

[1] Dr. Eshkevari, professor emeritus, Georgetown University Medical Center; and Founder of Aevsta Alternative Care, as first author,

conceptualized the idea, designed the research protocol, and led the research endeavor at the two clinical sites. She was the primary mentor for doctoral students, Collins, Donohue, Sales, and Totoraitis. She was also instrumental in obtaining IRB approval for the study. She was involved in the manuscript preparation and approved the final article submitted herein.

[2]Drs. Collins, Donohue, Sales, and Totoraitis were trainees in the Doctor of Nurse Anesthesia Practice Program, Georgetown University; and contributed to the research project by conducting literature reviews, assisting with IRB submission at Georgetown University, participating in statistical analysis and manuscript preparation. They have reviewed and approved the final manuscript.

[3]Dr. Carrie Bowman Dalley, Georgetown University Medical Center served as secondary mentor to the students, and in her role reviewed IRB submissions, and contributed greatly to manuscript preparation. She has approved the final article.

[4] Drs. Bregman and [5]Negro served as our psychiatry physician colleagues and experts. They were consulted on the study design and assisted with patient monitoring as needed. They have reviewed and approved the final manuscript.

[6] Ms. Stephanie Gordon and Mr. Christian Estrada served as medical assistants who recruited patients, assigned them randomly to groups to be evaluated, and worked diligently on data collection, and analysis together with our research students above. They have reviewed and approved the final manuscript.

All authors attest that the above statements are correct and accurate.

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Declaration of interest statement

All authors attest to: Declarations of interest: none

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CRediT authorship contribution statement

Ladan Eshkevari: Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Michelle Sales:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Christina Collins:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Julia Totoraitis:** Writing – original draft. **Lindsay Donohue:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Carrie Bowman-Dalley:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis. **Bejamin Bregman:** Supervision, Writing – review & editing. **Paulo Negro:** Writing – original draft, Supervision, Formal analysis. **Stephanie Gordon:** Writing – review & editing, Validation, Project administration, Data curation. **Christian Estrada:** Writing – review & editing, Visualization, Project administration, Data curation.

Declaration of competing interest

The authors declare no potential conflicts of interest

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