Biological and Social Determinants of Suicidal Behaviour

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ABSTRACT

Background: Suicide is a worldwide concern, claiming nearly one million lives each year. The causes of suicidal behaviour are unclear, but a variety of biological, psychological, social, and environmental factors are thought to contribute to suicide risk. Many cases of suicidal behaviour cannot be explained by conventional risk proposed by clinical and research observations. Recent research has focused on biomarkers of suicidal behaviour, including brain-derived neurotrophic factor (BDNF). This thesis aims to determine the association between BDNF and suicidal behaviour by reviewing the literature and by analyzing clinical data. An additional aim of this thesis is to explore the associations between social factors and suicidal behaviour, with a particular focus on sex differences within these factors.

Methods: We explored the associations between biological and social risk factors and suicidal behaviour in several ways. We conducted a systematic review to summarize and evaluate the literature regarding BDNF levels and suicidal behaviour. The protocol for this systematic review was designed and published a priori. We performed a qualitative review of the literature and a meta-analysis of studies of serum BDNF and attempted suicide. Then, we assessed the association between serum BDNF and attempted suicide using a case-control study design. We analyzed data collected from the Study of Determinants of Suicide Conventional and Emergent Risk (DISCOVER), and age- and sex-matched study of attempted suicide. In a sample of 250 participants (84 cases of attempted suicide, 104 psychiatric controls, and 93 community controls), we used linear regression analysis to determine the association between BDNF level and attempted suicide, adjusting for age, sex, body mass index, current smoking status, and

antidepressant use. Finally, using the same dataset, we explored the associations of a number of social factors with attempted suicide. In a sample of 343 participants (146 cases, 104 psychiatric controls, and 93 community controls), we used logistic regression analyses to determine the associations between social risk factors and attempted suicide in men and women separately. These included age, education level, employment status, marital status, religious practice, stressful life events, and childhood abuse.

Results: Our systematic review included 14 studies. The meta-analysis of three studies of serum BDNF and attempted suicide showed no significant association. The qualitative review of all studies revealed inconsistent findings regarding associations between BDNF and suicidal behaviour. In our study of serum BDNF and attempted suicide in the DISCOVER dataset, attempted suicide was not significantly associated with BDNF level. In our study of social factors for attempted suicide, some sex differences were found: Completion of post-secondary education and religious practice were found to be significant protective factors against attempted suicide only in women, and unemployment and stressful life events were significant risk factors only in men.

Conclusion: This thesis provides important findings about the biological and social risk factors for suicidal behaviour. Understanding the determinants of suicidal behaviour can aid clinicians in identifying and treating vulnerable individuals.

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I dedicate this work to those who have lost loved ones to suicide.

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LIST OF ABBREVIATIONS

AD: Adjustment disorder

BDNF: Brain-derived neurotrophic factor

BLA: Basolateral amygdala

BSS: Beck Scale for Suicidal Ideation

CeA: Central nucleus of the amygdala

CI: Confidence interval

CSF: Cerebrospinal fluid

DISCOVER: Determinants of Suicidal Behaviour: Conventional and Emergent Risk

ELISA: Enzyme-linked immunosorbent assay

GRADE: Grading of Recommendations, Assessment, and Evaluation

HDRS: Hamilton Depression Rating Scale

MDD: Major depressive disorder

MINI: Mini International Neuropsychiatric Interview

MOOSE: Meta-analysis of Observational Studies in Epidemiology

NOS: Newcastle-Ottawa Scale

OR: Odds ratio

PD: Personality disorder

PMI: Postmortem interval

PFC: Prefrontal cortex

PPAD: Postpartum affective disorder

PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses

RDD: Recurrent depressive disorder

Ref.: Reference category

SD: Standard deviation

SIOSS: Self-rating Idea of Suicide Scale

SMD: Standardized mean difference

SSI: Scale for Suicidal Ideation

TrkB: Tropomyocin receptor kinase B

VIF: Variance inflation factor

DECLARATION OF ACADEMIC ACHIEVEMENT

I am the primary author of each study included in this thesis. For each study I developed the research question and study protocol. I planned and performed all statistical analyses and wrote each manuscript. Detailed explanations of all authors' contributions are presented at the end of each study.

CHAPTER 1

1.1 BACKGROUND

Suicide has been identified by the World Health Organization as a leading cause of death worldwide. It claims nearly one million lives each year and the numbers continue to increase (1). Suicide has a devastating impact at individual, family, community, and societal levels. Suicidal behaviour encompasses a range of ideas, plans, and acts intended to end one's own life. Non-fatal suicidal behaviours occur 10-20 times as often as completed suicide and increase one's risk of dying by suicide (2). Following a non-fatal suicide attempt, individuals are at increased risk of mental and physical health problems, economic difficulties, and decreased quality of life (3).

Suicidal behaviour is a complex phenomenon for which the causes are unclear. However, research has identified a number of factors that may contribute to the risk of suicidal behaviour. These include internal factors (biological and psychological) and external (social and environmental) factors. Internal factors include psychiatric disorders (especially mood disorders), substance use disorders, chronic illness, and demographic variables (e.g. age and sex) (2, 4). External factors include employment status, marital status, adverse experiences, and living alone (4-6). Having a psychiatric disorder, particularly a mood disorder, is an especially important risk factor, and about 90% of individuals who attempt or complete suicide have a psychiatric disorder (4, 7). However, most individuals who have a psychiatric disorder never attempt suicide. This indicates that a predisposition toward suicidal behaviour may play an important role, independent of an underlying psychiatric disorder. As well, many cases of suicidal behaviour cannot

be explained by conventional risk factors proposed by clinical and research observations. Recently, the focus of research has shifted to the investigation of biological markers in suicide risk. These include genetic variants, neurotransmitters such as serotonin, and neurotrophins such as brain-derived neurotrophic factor (BDNF) (2, 8). Incidents of suicidal behaviour likely involve interactions among biological and social factors. Further investigation of the risk factors for suicidal behaviour will improve our understanding of this phenomenon and assist in prevention efforts.

1.2 THESIS OBJECTIVES

This thesis is composed of three studies that explore risk factors for suicidal behaviour. The first two studies focus on brain-derived neurotrophic factor (BDNF) as a possible biomarker for suicidal behaviour, while the third study focuses on social risk factors.

Chapters 2 and 3 describe a systematic review conducted to summarize and evaluate the literature regarding BDNF (central and peripheral levels) and suicidal behaviour (including suicidal ideation, attempts, and completion) (Study 1). Chapter 2 contains the protocol for the systematic review, including an a priori defined search strategy and a detailed plan for screening of studies, data extraction, risk of bias assessment, and statistical analysis. This protocol was published in *Systematic Reviews* (9). Chapter 3 contains the completed systematic review, in which observational studies of BDNF and suicidal behaviour were qualitatively and quantitatively summarized and critically assessed. This systematic review was also published in *Systematic Reviews* (10).

Chapter 4 presents a case-control study examining the association between serum BDNF level and attempted suicide (Study 2). The study sample comprises individuals

who have made a recent suicide attempt and both psychiatric inpatients and community members who have never attempted suicide. This manuscript has been accepted for publication in *Scientific Reports*.

Chapter 5 focuses on social risk factors for suicidal behaviour. In this study, sex differences are examined regarding the associations between a variety of social factors and attempted suicide (Study 3). Like in Chapter 4, individuals who have made a suicide attempt are compared to psychiatric and community controls. This manuscript has been submitted to *Biology of Sex Differences*.

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CHAPTER 2

Study 1: Part 1

Association between BDNF levels and suicidal behaviour: a systematic review protocol

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Keywords: Suicide, Attempted suicide, Suicidal ideation, Brain-derived neurotrophic factor, Systematic review, Protocol

Systematic review registration: PROSPERO CRD42015015871

See appendix 1 for published paper.

2.1 ABSTRACT

Background: Suicide is a worldwide public health concern that claims close to 1 million lives each year. Suicidal behaviour is a significant risk factor for completed suicide and is much more prevalent than completed suicide. Many internal and external factors contribute to the risk of suicidal behaviour. Recent research has focused on biological markers in suicide risk, including brain-derived neurotrophic factor (BDNF). BDNF is a protein involved in the growth, function, and maintenance of the nervous system. It has been implicated in psychiatric disorders and suicide. While some evidence suggests that reduced levels of BDNF are associated with suicide, the precise relationship has yet to be determined. The aim of this study is to review the literature examining the relationship between levels of BDNF and suicidal behaviour.

Methods: A predefined search strategy will be implemented to search the following electronic databases: PubMed/MEDLINE, Excerpta Medica Database (EMBASE), PsycINFO, and Cumulative Index to Nursing and Allied Health Literature (CINAHL) from inception. The articles will be screened by two independent authors (RE and SP) using predetermined inclusion and exclusion criteria. Discrepancies will be resolved by consensus, or by a third author (ZS) in cases of disagreement. The primary outcome will be the association between levels of BDNF and suicidal behaviour. A meta-analysis will be conducted if appropriate. Quality of evidence and risk of bias will be evaluated.

Discussion: The findings of this review will assist in identifying and treating individuals at increased risk of suicide.

2.2 BACKGROUND

Suicide is a worldwide public health concern. It claims the lives of over 800,000 people each year, and the numbers continue to increase (1). Suicide has a devastating impact at a number of levels, including the individual, family, community, and society. Suicidal behaviour encompasses a complex set of ideas, plans, and acts intended to end one's own life. It occurs 10 to 20 times more often than completed suicide, and is a significant risk factor for completed suicide in the general population (1, 2).

While the causes of suicide are unclear, a number of internal (biological and psychological) and external (social and environmental) factors are thought to contribute to the risk of suicidal behaviour. Internal risk factors include psychiatric disorders (especially mood disorders), substance use disorders, chronic illness, and demographic variables (age and sex) (3). External factors include unemployment, unmarried status, and a lack of social support (2, 3). While having a psychiatric disorder significantly increases one's risk of suicide (4), and about 90% of people who attempt or complete suicide have a psychiatric disorder (3), most individuals with psychiatric disorders never attempt suicide. This indicates that there may be a predisposition toward suicidal behaviour independent of the underlying psychiatric disorders (2). As well, many cases of suicide cannot be explained by the conventional risk factors proposed by clinical and research observations. Therefore, the focus has shifted to the investigation of biological markers in suicide risk, which has become more common among the recent literature.

The role of neurotrophins has been explored in relation to psychiatric disorders, including depression, bipolar disorder, anxiety, and schizophrenia (5). Neurotrophins are a family of proteins that regulate the survival, development, maintenance and function of

vertebral nervous systems (6). Brain-derived neurotrophic factor (BDNF) is the most abundant member of the neurotrophin family (6) and has been implicated in both suicide and suicidal behaviour (7). BDNF is expressed in the brain and in other body tissues such as skeletal muscle and circulates throughout the body in the bloodstream (7, 8). When BDNF is released by a cell, it triggers a cascade of events that lead to neurogenesis, nerve growth, neuroplasticity, and neurotransmission (7). BDNF is also important in morphological plasticity, neurite outgrowth, phenotypic maturation, and protein synthesis for neuron and synaptic functioning (6). Since BDNF is intrinsic to these important processes, pathological changes in BDNF levels are likely involved in neurological deficits that impair one's ability to adapt to difficult situations. Altered levels of BDNF may be responsible for the cognitive deficits and altered brain structure associated with depression, stress, and suicide.

Some studies have linked reduced levels of BDNF to psychiatric disorders and suicide. Low levels of serum BDNF are associated with a dispositional vulnerability to depression and with acute depressive states in the general population (9). Lower levels of both serum and plasma BDNF are associated with major depressive disorder, and serum levels in particular have been correlated to severity of depression (10, 11). Stress, which plays an important role in suicidal behaviour and constitutes a major risk factor (7, 12), is associated with altered levels of BDNF in the brain (13-16). But while brain levels of BDNF are altered in depression and stress, evidence suggests a differential role of BDNF depending on the location in the brain. Depression and stress are associated with low levels of BDNF in the hippocampus and prefrontal cortex but high levels of BDNF in the

amygdala and nucleus accumbens (17). Antidepressants have been shown to normalize levels of BDNF expression (18).

Postmortem studies of the brains of suicide victims have revealed abnormally low levels of BDNF and its receptor, tropomyocin receptor kinase B (TrkB), compared to controls (19, 20). Interestingly, this was true regardless of the psychiatric diagnosis. Other studies have measured peripheral levels of BDNF in the blood of suicidal individuals. Deveci and colleagues compared serum BDNF levels among suicide attempters, depressed patients, and healthy controls (21). Mean serum BDNF was significantly lower in both the suicidal group and the depressed group compared to controls. Studies of plasma BDNF have found decreased levels in suicidal depressed patients compared with nonsuicidal depressed patients (22, 23).

BDNF is a modifiable risk factor for suicidal behaviour. However, relatively few studies have investigated the relationship between levels of BDNF and suicidal behaviour. As well, since BDNF levels are altered in both depression and suicide, it is unclear whether the differences are related specifically to suicide. A systematic review is needed in order to summarize the existing studies and determine whether BDNF levels are in fact associated with suicidal behaviour, as well as to identify gaps in the literature that require further research.

2.2.1 Objectives

The objective of this systematic review is to elucidate the association between levels of BDNF and suicidal behaviour (including completed suicide, attempted suicide, and suicidal ideation) in an adult population through a methodological summary of the literature.

The study goals are the following:

1. To investigate the relationship between levels of BDNF and suicidal behaviour by summarizing primary studies that have examined this relationship.
2. To combine the results of primary studies in a statistical manner using meta-analysis, when appropriate.
3. To critically evaluate the existing literature and identify which areas require additional research.

2.3 METHODS/DESIGN

2.3.1 Inclusion and exclusion criteria

This systematic review will include published observational studies (case-control and cohort studies) of central and peripheral levels of BDNF (including postmortem brain tissue, cerebrospinal fluid, plasma, serum, whole blood, and urine) and suicidal behaviours (including completed suicide, attempted suicide, and suicidal ideation), in a population aged 18 and older. Included studies will have investigated the association between levels of BDNF and suicidal behaviour by comparing BDNF levels between groups with and without suicidal behaviour. This review will include clinical samples as well as community-based samples. No demographic limitations will be applied apart from age, and no special populations will be excluded (e.g. incarcerated individuals, pregnant women, etc.).

2.3.2 Search strategy

All relevant studies will be identified with no language or time restrictions. The databases to be searched from inception are as follows: PubMed/MEDLINE, PsycINFO,

Excerpta Medica Database (EMBASE), and Cumulative Index to Nursing and Allied Health Literature (CINAHL). The search strategy (presented in Table 2.3.2) will use relevant keywords and their associated medical subject headings (MeSH). A number of different search terms related to suicidal behaviour that are common in the literature will be used in order to encompass this broad topic, including "suicide", "attempted suicide," "self-injurious behaviour", "self harm*," "automutilation," "self inflict*," and "suicidal ideation." These terms will be combined with the term "brain-derived neurotrophic factor" or "BDNF". The search will include titles, abstracts, and keyword fields. The reference lists from the included articles will be scanned manually to identify additional studies. The grey literature will be searched using the ProQuest Dissertations and Theses Database. Reviews, abstracts, and commentaries will be excluded. No language restrictions will be applied. An experienced health sciences librarian (LB) was consulted and assisted in the search strategy. A search alert will be set up to ensure the retrieval of relevant studies published after the initial search.

2.3.3 Data screening

All citations and abstracts retrieved using the predefined search strategy (Table 2.3.2) will be screened by two raters (RE and SP) independently. Eligible articles will be identified using pre-established criteria and retrieved for full-text review. Disagreements at any point in the review process will be resolved by discussion. In cases where consensus is not reached, eligibility will be determined by a third author (ZS). Studies that are ineligible will be excluded from review. The reasons for exclusion will be recorded and described in the flow diagram (Figure 2.3.3). For each phase of screening,

the Kappa statistic will be used to calculate inter-rater agreement (24). The authors of the studies will be contacted for clarification and additional data when necessary.

2.3.4 Data extraction

The two raters (RE and SP) will extract data independently from the included studies using a pre-established data extraction form that will be pilot-tested beforehand. The raters will obtain the following information from each study: first author, year of publication, city and country of publication, article title, journal, study design, description of sample population, mean age, ethnicity, and definition of suicidal behaviour. For studies that include more than one measure of suicidal behaviour, each measure will be recorded. This will allow for the combination of studies with the same measures in a meta-analysis. For example, some studies have used the suicide item in Hamilton Depression Rating Scale (HDRS), while others have used the Beck Scale for Suicidal Ideation (BSS) (25, 26). We will record all measures reported so that we can combine, for instance, all studies that used the BSS. We expect most studies to report suicidal behaviour as a dichotomous measure (e.g. history of suicide attempt or no history of suicide attempt). However, if studies used different measurement scales to indicate severity of suicidal behaviour, then we will use a dichotomized outcome based on the presence of suicidal behaviour, regardless of severity. For studies that report multiple time points for suicidal behaviour (e.g. suicide attempt within the last month, vs. the past 3 months, vs. lifetime), all time points will be recorded. This will allow for the combination of similar time frames when possible. Information regarding the BDNF measurements will be obtained, including the tissue sample in which it was measured, the lab analysis methods, the mean BDNF levels and standard deviations, and the unit of

measurement used. If relevant, the comparison group and any underlying psychiatric disorders will also be recorded. For each study, primary and secondary outcome measures, results, statistical analyses, and conclusions will be recorded. If any data are missing or incomplete, authors will be contacted for additional details.

2.3.5 Assessment of quality

The risk of bias of included studies will be assessed by two independent raters (RE and SP) using the Newcastle-Ottawa Scale (NOS) (27). An adapted version of the NOS will be used, in keeping with previous systematic reviews of observational studies (28). This version of the NOS contains seven questions in the following domains: methods for selecting study participants (selection bias), methods to control for confounding (performance bias), statistical methods (detection bias), and methods of exposure and outcome assessment (information bias). Risk of bias will be assessed on a scale from 0 to 3, where 0 indicates high risk of bias and 3 indicates low risk. Descriptions and examples of high and low risk of bias are provided. This adapted NOS also includes categories related to statistical methods, confounding effects, and reporting of missing data. The Grading of Recommendations, Assessment, and Evaluation (GRADE) framework will be used to report the quality of evidence and strength of recommendations (29). This framework provides a systematic approach for considering and reporting risk of bias, imprecision, inconsistency, indirectness of study results, and publication bias. A summary of findings table will be presented to allow for assessment of confidence in the estimates.

2.3.6 Statistical analyses and heterogeneity

The results of this systematic review will be presented as a qualitative summary of the literature. When possible, meta-analyses will be performed. This review will encompass a wide variety of studies with different designs, sample populations, BDNF measurements, and definitions of suicidal behaviour. Clinical and methodological heterogeneity are expected. Therefore, separate meta-analyses will be conducted on groups of studies that share the following characteristics:

1. Study design (e.g. case control vs. cohort)
2. Definition of suicidal behaviour (completed suicide, attempted suicide, or suicidal ideation)
3. Type of tissue from which BDNF was sampled (e.g. plasma, serum, brain tissue)

Meta-analyses will be performed using the extracted data from groups of studies if the following conditions are met:

- More than one study is found that share all of the characteristics listed above
- There are minimal differences among the studies in other relevant characteristics (such as sample population)
- Data in each study are available and reported with sufficient detail.

Heterogeneity will be assessed using the I^2 statistic. The interpretation of the I^2 value will be based on the guidelines in the Cochrane Handbook for Systematic Reviews of Interventions, which defines 0% to 40% as low heterogeneity, 30% to 60% as moderate heterogeneity, 50% to 90% as substantial heterogeneity, and 75% to 100% as considerable heterogeneity (30). In this study, an I^2 value below 50% will be considered low heterogeneity. The P value from the chi-squared test will also be taken into

consideration, with significant heterogeneity being defined with a P value below 0.10. Groups of studies in which heterogeneity is found to be low ($I^2 < 50\%$) will be assessed in a combined statistical manner using meta-analysis. The mean differences (MD) in BDNF level between groups with and without suicidal behaviour will be combined into a summary estimate. Only adjusted values extracted from the primary studies will be used. A random-effects model will be implemented, as it accounts for both within-study and between-study variability. As well, a mixed-effects model will be used to examine the possible mediation effect of BDNF on the relationship between other variables (including sex, age, and psychiatric diagnosis) and risk of suicidal behaviour. Sensitivity analysis will be conducted based on risk of bias; studies with a score of 0 on the NOS will be excluded to determine whether the summary estimate stays the same.

The main source of heterogeneity hypothesized is clinical heterogeneity, resulting from diversity in the populations being studied. Some studies have derived their samples from particular clinical populations, such as depressed patients, while others have sampled populations with a range of psychiatric diagnoses, or community-based populations. Since alterations in BDNF levels are linked to psychiatric disorders, particularly depression, the sample characteristics could have a significant influence on the associations between BDNF levels and suicidal behaviour (5, 9). The implications of this heterogeneity on the interpretation of the results will be discussed.

In the event that the heterogeneity is too high to allow for meta-analyses to be performed, the results of this systematic review will be presented as a narrative summary of the literature examining the relationship between levels of BDNF and suicidal

behaviour. The included studies will be synthesized in a comprehensive, up-to-date review of this emerging area of research.

2.3.7 Presenting and reporting of results

This systematic review will be performed and presented according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, as well as the Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines (31, 32). The article selection process will be summarized in a flow diagram (see Figure 2.3.3). The relevant outcomes and characteristics of each study will be reported in summary tables. Publication bias will be assessed using Egger's plot.

This protocol follows the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) 2015 Statement (33).

2.4 DISCUSSION

This systematic review will present evidence from which conclusions can be made regarding the relationship between levels of BDNF and suicidal behaviour. It is expected that an inverse correlation will be found, with reduced levels of BDNF associated with suicidal behaviour. The findings of this systematic review will contribute to our understanding of BDNF as a biological factor involved in suicide risk, and of suicide pathology more generally. These findings, as well as the appraisal of the status of the literature, will be of use to clinicians, in identifying individuals at increased risk of suicide, and researchers, in developing therapeutic targets.

2.5 ACKNOWLEDGEMENTS, COMPETING INTERESTS, AND AUTHOR CONTRIBUTIONS

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The authors declare that they have no competing interests.

RE conceived and designed the study, wrote and revised critically the manuscript, devised the data extraction form, and approved the final manuscript. SP assisted in drafting the manuscript; participated in the methodology, interpretation, and critical revision of the manuscript; and approved the final manuscript. MB participated in the critical revision, developed the quality assessment tool, and approved the final manuscript. LB participated in the development of the search strategy and approved the final manuscript. RA participated in the critical revision and methodology and approved the final manuscript. LM participated in the critical revision and methodology and approved the final manuscript. ZS conceived and designed the study, participated in the methodology and critical revision, and approved the final manuscript. All authors read and approved the final manuscript.

2.6 FIGURES AND TABLES

Figure 2.3.3: PRISMA Flow Diagram

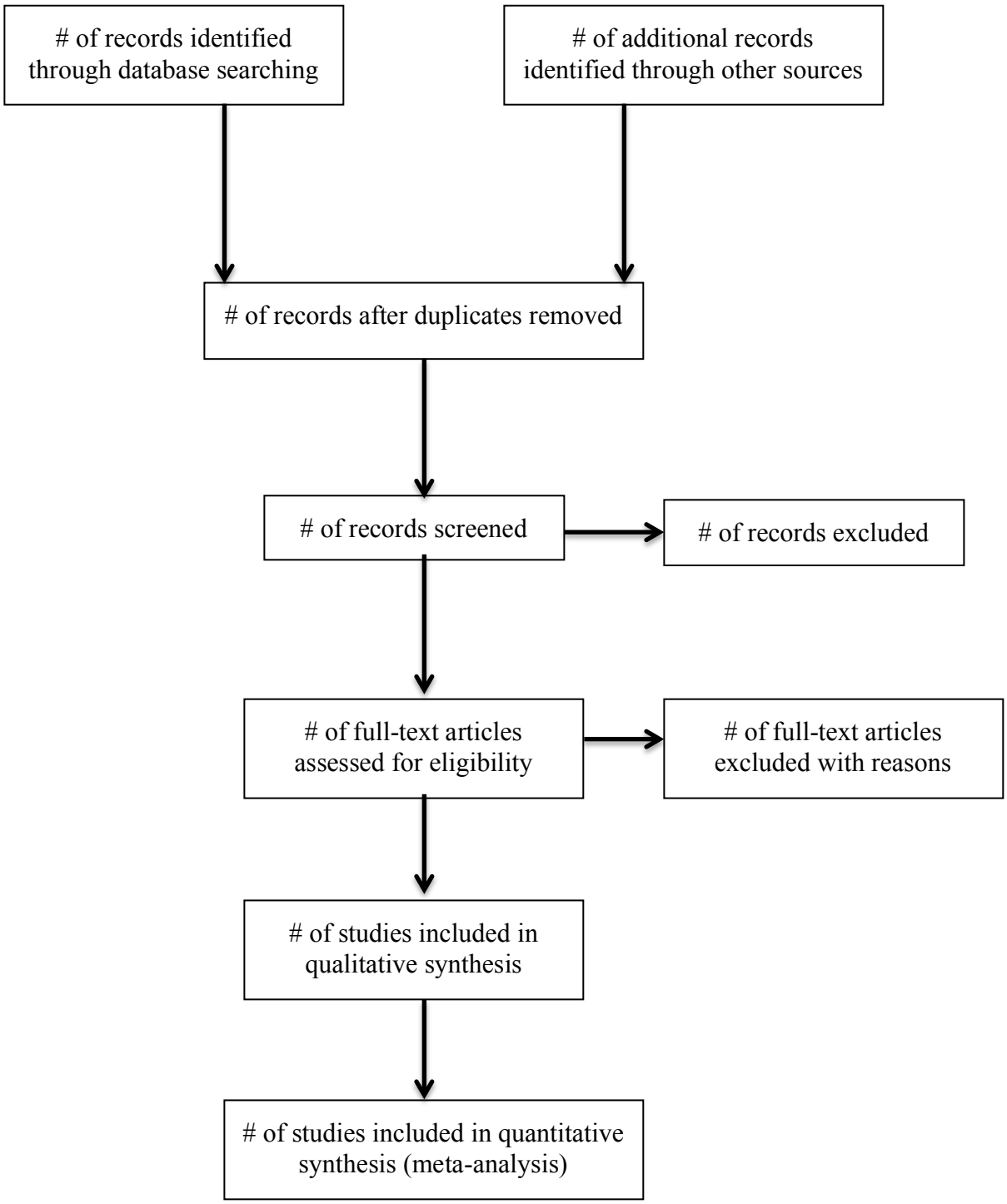

Table 2.3.2: Search Strategy

Database	Search Strategy
MEDLINE (n=106)	1. exp Suicide/ 2. suicid*.mp. 3. exp Self-Injurious Behavior/ 4. (self harm* or self inflict* or self injur* or self wound* or self mutilat*).mp. 5. automutilat*.mp 6. 1 or 2 or 3 or 4 or 5 7. brain derived neurotrophic factor.mp. or Brain-Derived Neurotrophic Factor/ 8. bdnf.mp 9. 7 or 8 10. 6 and 9
EMBASE (n=366)	1. exp suicidal behavior/ 2. suicid*.mp. 3. exp automutilation/ 4. (self harm* or self inflict* or self wound* or self mutilat* or autmutilat*).mp. 5. 1 or 2 or 3 or 4 6. brain derived neurotrophic factor.mp. or exp brain derived neurotrophic factor/ 7. bdnf.mp. 8. 6 or 7 9. 5 and 8
PsycINFO (n=76)	1. exp Suicide/ 2. exp Attempted Suicide/

	3. exp Suicidal Ideation/ 4. suicide*.mp 5. exp Self Injurious Behavior/ 6. (self harm* self injur* or self inflict* or self wound* or self mutilat* or autmutilat*).mp. 7. 1 or 2 or 3 or 4 or 5 or 6 8. brain derived neurotrophic factor.mp. or exp Brain Derived Neurotrophic Factor/ 9. bdnf.mp. 10. 8 or 9 11. 7 and 10
CINAHL (n=4)	1. MH (“Suicide+”) 2. “suicid*” 3. MH (“Self-Injurious Behavior”) 4. "self harm*" OR "self injur*" OR "self inflict*" OR "self wound*" OR "self mutilat*" OR "automutil*" 5. 1 or 2 or 3 or 4 6. “brain derived neurotrophic factor” 7. “bdnf” 8. 6 or 7 9. 5 and 8

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CHAPTER 3

Study 1: Part 2

Association between BDNF levels and suicidal behaviour: A systematic review and meta-analysis

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See appendix 2 for published paper.

3.1 ABSTRACT

Background: Suicidal behaviour is a complex phenomenon with a multitude of risk factors. Brain-derived neurotrophic factor (BDNF), a protein crucial to nervous system function, may be involved in suicide risk. The objective of this systematic review is to evaluate and summarize the literature examining the relationship between BDNF levels and suicidal behaviour. **Methods:** A predefined search strategy was used to search MEDLINE, EMBASE, PsychINFO, and CINAHL from inception to December 2015. Studies were included if they investigated the association between BDNF levels and suicidal behaviours (including completed suicide, attempted suicide, or suicidal ideation) by comparing BDNF levels in groups with and without suicidal behaviour. Only observational were included (case-control and cohort studies). Both clinical and community-based samples were included. Screening, data extraction, and risk of bias assessment were conducted in duplicate. **Results:** Six hundred thirty-one articles were screened and 14 were included in the review. Three studies that assessed serum BDNF levels in individuals with suicide attempts and controls were combined in a meta-analysis that showed no significant association between serum BDNF and suicide attempts. The remaining 11 studies were not eligible for the meta-analysis and provided inconsistent findings regarding associations between BDNF and suicidal behaviour. **Conclusion:** The findings of the meta-analysis indicate that there is not a significant association between serum BDNF and attempted suicide. The qualitative review of the literature did not provide consistent support for an association between BDNF levels and suicidal behaviour. The evidence has significant methodological limitations.

3.2 BACKGROUND

Suicide is a growing public health concern. Worldwide, over 800,000 people die by suicide every year, and the numbers are increasing (1). Suicide affects not only the individual but the family, community, and society in which it occurs. Non-fatal suicidal behaviours, which refer to a complex set of thoughts, plans, and acts intended to end one's life, are a significant risk factor for completed suicide and occur 10-20 times more often than completed suicide (1, 2).

A multitude of factors are thought to contribute to the risk of suicidal behaviour, including internal (biological and psychological) and external (social and environmental) factors. Examples of internal risk factors include psychiatric disorders, substance use disorders, chronic illness, and demographic variables (such as older age and female sex) (3). External risk factors can include unmarried status, unemployment, and a lack of social support (2, 3). Most suicidal behaviour occurs in the context of a psychiatric disorder (90% of attempted or completed suicides), but most individuals with psychiatric disorders never attempt suicide (3, 4). In addition, many cases of suicide cannot be explained by the conventional risk factors that have been proposed by research and clinical observations. Consequently, there is a need to identify predictors of suicidal behaviour beyond the known risk factors (2).

Recent research has focused on biological markers of suicide risk, such as genetic variants and circulating proteins (5). One such protein is brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family of proteins. BDNF is found in the brain and throughout the body in the bloodstream (6). It is crucial to a number of neural processes, such as neurogenesis, neuroplasticity, and neurotransmission (6, 7).

Altered BDNF levels may play a role in the pathogenesis of suicidal behaviour by resulting in long-term changes in the brain that can lead to neuropsychological deficits. A number of studies have shown that changes in brain structure and function may be associated with depression, stress, and suicidal behaviour. These changes include reductions in neuron cell number, density, and size, as well as decreased cortical thickness and changes in synaptic circuitry (8-11). Other studies demonstrate cognitive deficits in stress and depression (12). This evidence supports a new hypothesis that links the pathogenesis of suicidal behaviour and depression to altered neural plasticity, which impairs the brain's ability to respond appropriately to environmental stimuli (13, 14). It is hypothesized that pathological changes in BDNF levels are distally responsible for these neuropsychological deficits associated with depression, stress, and suicide (6).

It is also possible that short-term changes in BDNF levels may be involved in suicidal behaviour pathogenesis. There is evidence that BDNF levels can undergo short-term variations in response to external stimuli. Serum BDNF levels have been shown to increase following a 3-month reduced-calorie diet (15) and endurance training (16). Antidepressant treatment in depressed individuals normalizes low levels of BDNF (17). Alcohol and tobacco use have also been linked to altered levels of BDNF; excessive drinkers tend to have lower serum BDNF levels, and current smokers tend to have higher serum BDNF levels (18). These and other variables may explain variations in BDNF level between individuals and within individuals over time. These factors may also be related to risk of suicidal behaviour, and could explain how BDNF might be related to suicidal behaviour in a proximal manner. However, there is no conclusive evidence linking short-term changes in BDNF to suicidal behaviour.

While BDNF is primarily produced in the central nervous system, it is also expressed in peripheral tissue in smooth muscle cells, endothelial cells, endocrine cells, and immune cells (18). It has been shown to cross the blood-brain barrier, and blood levels of BDNF are reflective of brain levels (19). Brain levels of BDNF can be measured in postmortem brain tissue, and circulating levels can be measured in the blood (serum or plasma) and cerebrospinal fluid (CSF) of living individuals.

Altered central and peripheral BDNF levels have been implicated in both depression (20-22) and stress (23-26), both of which are risk factors for suicidal behaviour (6). Furthermore, altered BDNF levels have been linked to suicidal behaviour in postmortem brain studies (27, 28). Clinical studies have shown reduced peripheral BDNF levels in both the serum and plasma of suicidal individuals (29-31). While this is a growing area of research, the relationship between BDNF levels and suicidal behaviour remains unclear, as relatively few studies have explored this relationship. In addition, since some of these studies have examined recent suicidal behaviour while others examined lifetime suicidal behaviour, it is uncertain whether BDNF is related to suicide in a distal or proximal manner. To date, there has not been a systematic review undertaken to summarize the literature.

This paper aims to systematically evaluate and summarize the existing literature relating BDNF levels (including central and peripheral levels) to suicidal behaviour (including completed suicide, attempted suicide, and suicidal ideation) in adult populations. Based on our current understanding of BDNF and its role in brain structure and function, it is expected that low BDNF levels will be associated with suicidal behaviour in studies of both central and peripheral BDNF levels.

3.3 METHODS

3.3.1 Search strategy

The protocol for this systematic review was published previously (32). This systematic review follows the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines as well as the Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines. An a priori defined search strategy was developed with the help of an experienced Health Sciences librarian (LB) and was used to search the following databases from inception until December 2015: PubMed/MEDLINE, PsychINFO, EMBASE, and CINAHL. The search strategy can be found in the published protocol. One amendment was made to the original search strategy. Because many cases of suicidal behaviour involve overdose of substances, two search terms ("self poison*" and "overdose") were added to capture studies of those behaviours. An example of the search strategy for MEDLINE is presented in Table 3.3.1. The grey literature was searched for previously published theses using the ProQuest Dissertations and Theses A&I database. The reference lists of included articles were scanned manually.

3.3.2 Inclusion and exclusion criteria

This review included observational studies that investigated associations between levels of BDNF (central or peripheral, including postmortem brain tissue, cerebral spinal fluid, and blood) and suicidal behaviours (including completed suicide, attempted suicide, and suicidal ideation) in adult populations (aged 18 and older). Studies of both clinical and community-based samples were included. No demographic limitations were applied apart from age.

3.3.3 Screening and data extraction

Two raters (RE and SP) screened the articles identified by the literature search. Articles were screened first by title, then by abstract for full-text review. Studies that met the inclusion criteria upon full-text review were identified for data extraction. For articles that were excluded, reasons for exclusion were documented. Discrepancies at any point in the screening process were resolved by discussion, and in cases of disagreement a third rater (ZS) was consulted. The Kappa statistic was used to calculate inter-rater agreement (33).

Two independent raters (RE and SP) performed the data extraction in duplicate. A pre-established, pilot-tested data extraction form was used. The following information was obtained from each study: study information (title, author, publication year, journal name, location of study), study setting and design, description of sample and comparison groups, sample size, definition and measurement of suicidal behaviour, type of BDNF sample, statistical methods, mean BDNF levels and standard deviations, and limitations. Four authors were contacted for further information and one responded with numerical data not presented in the article. One article was published in Mandarin (34), so a fluent Mandarin speaker assisted with the determination of its eligibility and data extraction. Risk of bias in included studies was assessed in duplicate using an adapted version of the Newcastle Ottawa Scale, which assesses for risk of selection bias, performance bias, detection bias, and information bias (35).

3.4 RESULTS

3.4.1 Search results

The database search retrieved 631 records. After the removal of duplicates, 488 titles were screened and 438 records were excluded. An additional 30 were excluded upon the review of the abstracts. 20 full-text articles were assessed for eligibility. Of these, 14 were included in this review (see Figure 3.4.1 for PRISMA flow diagram). Inter-rater agreement for the title, abstract, and full-text screening was 0.59, 0.71, and 0.91 respectively, corresponding to fair, good, and excellent agreement (36).

3.4.2 Study characteristics

The characteristics of the included studies are summarized in Table 3.4.2. Twelve of the included articles were case-control studies and two were cross-sectional studies. Four of the studies were postmortem studies that measured BDNF in brain tissue samples from individuals who had died by suicide. The remaining nine studies were clinical studies, eight of which investigated blood levels of BDNF (serum or plasma) in participants who attempted suicide, and one of which investigated cerebrospinal fluid (CSF) levels of BDNF in individuals who experienced suicidal ideation.

3.4.3 Risk of bias assessment

The modified version of the Newcastle Ottawa Scale contains seven questions that fall under four domains: methods for selecting study participants (selection bias), methods to control for confounding (performance bias), statistical methods (detection bias), and methods of exposure and outcome assessment (information bias). Assessing the included studies using the modified Newcastle Ottawa Scale revealed a number of common sources of risk of bias. Nearly all of the studies (12/14) had samples sizes that were small, likely resulting in insufficient power to detect meaningful differences in mean BDNF level between groups. Most of the studies (10/14) compared groups of

between 20-30 participants, though some groups were as small as 10 participants. Another significant source of high risk of bias was the lack of adjustment for confounding variables. While some studies matched participants on age and/or sex in an attempt to reduce confounding, only four of the studies adjusted for any variables in their analyses. Finally, twelve of the fourteen studies used statistical methods that are inappropriate for observational studies. Univariate analyses were used to compare mean BDNF levels between groups. Only two studies (37, 38) performed a regression analysis to investigate the relationship between BDNF levels and suicidal behaviour.

3.4.4 Postmortem brain studies of completed suicide

Four of the included studies examined protein levels of BDNF in the brains of postmortem subjects (28, 27, 39, 40) (see Table 3.4.2). These studies employed a case-control design to compare protein levels in individuals who died by suicide with levels in non-suicide deaths. The studies have a combined total of 90 cases and 88 controls. Dwivedi et al. (2003) (27) used the Western blot technique to determine the protein levels of BDNF in prefrontal cortex (PFC) and hippocampal samples from 27 individuals who died by suicide and 21 non-psychiatric control subjects. They found significant differences in BDNF expression between the groups in both brain regions, with lower levels in the individuals who died by suicide. The authors assert that these differences were unrelated to psychiatric diagnosis or other measured variables (postmortem interval, brain pH, age, and sex). In a similar study, Karege et al. (2005) (28) compared BDNF levels in the ventral prefrontal cortex, hippocampus, and entorhinal cortex between 30 individuals who died by suicide and 24 non-psychiatric controls. The group of individuals who died by suicide was subdivided into three groups by diagnosis and

toxicology: untreated depressed, untreated other psychiatric disorder, and drug-treated depressed. The enzyme-linked immunosorbent assay (ELISA) technique was used to quantify BDNF in the tissue samples. Significantly reduced BDNF levels were found in both of the non-treated suicide groups compared to the non-suicide group, in the PFC ($p<0.002$) and hippocampus ($p<0.001$), but not in the drug-treated suicide group. No significant differences were found in the entorhinal cortex of any group. The third study to look at postmortem hippocampal levels of BDNF was conducted by Banerjee et al. (2013) (39). They also employed the ELISA method to compare BDNF levels between 21 individuals who died by suicide and 19 non-psychiatric controls. A significant difference was found between the groups, with reduced BDNF levels in individuals who died by suicide ($p<0.001$). The final study to examine brain levels of BDNF focused exclusively on the amygdala. Maheu et al. (2013) (40) measured BDNF levels in the basolateral amygdala (BLA) and central amygdala (CeA) of depressed individuals (22 and 25 respectively), and 14 healthy controls. Eleven of the depressed subjects from whom the BLA was sampled died of suicide and twelve of the depressed subjects from whom the CeA was sampled died of suicide. No significant differences were found between mean BDNF levels in individuals who died by suicide compared to controls.

3.4.5 Cerebrospinal fluid BDNF levels and attempted suicide

Only one study examined the association between BDNF in the CSF and suicidal behaviour. Martinez et al. (41) compared levels of proinflammatory and "resiliency" proteins (among them BDNF) between 18 depressed individuals and 25 healthy controls. While the mean BDNF levels were not presented or compared between suicidal and non-suicidal groups, the correlation between BDNF concentration and score on the Scale for

Suicidal Ideation (SSI) was calculated for 12 participants. A significant positive correlation was found between BDNF concentration and SSI score (r=0.62, p=0.033).

3.4.6 Serum BDNF levels and attempted suicide

Two of the included studies were cross-sectional studies that investigated serum levels of BDNF in clinical sample populations. These studies collectively assessed 241 individuals. Park et al. (2014) (42) conducted a pilot study relating serum BDNF levels to illness severity, suicide attempts, and central serotonin activity in depressed patients. The patients were stratified into subgroups based on their history of suicide attempts: 18 had a history of suicide attempts and 33 did not. Mean BDNF levels did not differ significantly between the two groups (p=0.3). The other cross-sectional study was conducted by Pinheiro et al. (2012) (38) in postpartum women. Of the 190 women included, 12 had a history of suicide attempts. No significant difference was found between mean BDNF levels in this group compared to the women with no history of suicide attempt (p=0.6). However, in women with postpartum affective disorder (n=29), suicide risk, as measured with the suicidality section of the Mini International Neuropsychiatric Interview (MINI), was significantly associated with lower BDNF levels (p=0.02).

The remaining four studies of serum BDNF and suicidal behaviour were case-control in design. The studies include a combined total of 148 cases and 335 controls. Two studies (29, 34) compared individuals with suicide attempts to both psychiatric and healthy controls. Deveci et al. (2007) (29) recruited 10 individuals with suicide attempts, 24 non-suicidal depressed individuals, and 26 healthy controls. Serum BDNF levels were found to be significantly lower in both the suicide group and the depressed group compared to the healthy control group (p=0.004). However, there was no significant

difference between BDNF levels in the suicide and depressed groups. Liang et al. (2012) (34) conducted a study comparing BDNF levels in depressed patients, with and without a history of suicide attempts, and healthy controls. The sample consisted of 31 depressed individuals with suicide attempts, 34 depressed individuals without suicide attempts, and 30 healthy controls. Serum BDNF levels were significantly different among the three groups, with the lowest levels in the suicide group ($p<0.01$). Among the 65 depressed individuals, BDNF levels were negatively correlated with scores on the Self-rating Idea of Suicide Scale (SIOSS) ($p<0.01$) (34).

The final two studies of serum BDNF levels focused on specific psychiatric disorders. Huang and Lee (2006) (43) measured BDNF levels in a group of 126 patients with schizophrenia, 11 of which had a history of suicide attempts. No significant difference in mean BDNF level was found ($p=0.841$). In a study by Grah et al. (2014) (37), associations between BDNF levels and suicidal behaviour were explored in patients suffering from depression, personality disorders, and adjustment disorders. The study included 51 patients with recurrent depressive disorder, 26 of which were suicidal; 59 patients with personality disorders, 33 of which were suicidal; 62 patients with adjustment disorders, 37 of which were suicidal; and 60 healthy controls. Significantly lower BDNF levels were found in those with suicide attempts in the personality disorder and adjustment disorder groups ($p=0.003$, $p=0.009$, respectively), but not in the depressed group.

A meta-analysis was performed using the results of three case-control studies that compared serum BDNF levels between suicide attempters and psychiatric controls (29, 43, 34) (Figure 3.4.6). These studies were selected for inclusion in the meta-analysis

based on their similar study designs (all case-control studies), definitions of suicidal behaviour (attempted suicide), and comparison groups (psychiatric controls). A random-effects model was used. The pooled estimate revealed a standardized mean difference (SMD) of -0.32 (95% CI –1.01 to 0.37), which corresponds to a small effect size according to Cohen's criteria (44). However, this estimate was not significant (p=0.36) and was associated with substantial heterogeneity (I^2=73%, p=0.02).

3.4.7 Plasma BDNF levels and attempted suicide

Three case-control studies measured plasma levels of BDNF in depressed individuals with and without a history of suicidal behaviour (30, 31, 45). These studies collectively assessed 80 cases and 246 controls. Kim et al. (2007) (30) compared 32 depressed patients hospitalized for recent suicide attempts to 32 hospitalized non-suicidal depressed patients and 30 healthy controls. They found significantly reduced plasma BDNF levels in suicide attempters compared to both control groups (p=0.009, p=0.008, respectively). Lee et al. (2007) (31) measured plasma BDNF levels in 77 hospitalized depressed patients (subdivided into 28 with a suicide attempt and 49 without a suicide attempt) and 95 healthy controls. This study also found a significant difference between BDNF levels in suicidal vs. non-suicidal individuals, with lower levels in the suicidal depressed group compared to the non-suicidal depressed group. Lee and Kim (2009) (45) conducted a study similar to Kim et al.'s (30) in which 20 hospitalized depressed individuals with recent suicide attempts were compared to 20 hospitalized non-suicidal depressed patients and 20 healthy controls. BDNF was measured in platelet-rich plasma, platelet-poor plasma, and platelets. In all three types of sample, BDNF levels were significantly lower in depressed patients (suicidal and non-suicidal) compared to healthy

controls, but no significant differences were found between suicidal and non-suicidal groups.

3.5 DISCUSSION

This systematic review aimed to evaluate and summarize the existing literature on associations between BDNF levels and suicidal behaviour. The 14 studies included in this review describe comparisons of mean BDNF levels between groups of individuals with and without suicidal behaviour. The definitions of suicidal behaviour, the methods of measuring BDNF level, and the sample populations, vary widely. The studies differ in their findings and methodological quality, producing an unclear picture of the relationship between BDNF levels and suicidal behaviour.

3.5.1 Postmortem brain studies of completed suicide

The postmortem studies of BDNF levels and completed suicide have examined multiple brain regions, including the hippocampus, prefrontal cortex, entorhinal cortex, and amygdala. Three studies (27, 28, 39) measured BDNF protein levels in the hippocampus and all found significant associations with completed suicide, suggesting that individuals who die by suicide have lower levels of BDNF. Two of those studies (27, 28) also measured BDNF levels in the PFC and found significant inverse associations with completed suicide. In the other brain regions studied, the entorhinal cortex (28) and the amygdala (40), no significant differences were found.

Of the four studies of brain BDNF levels in people who died by suicide, only one, Maheu et al. (40), included both psychiatric and non-psychiatric controls. The other three studies compared individuals who died by suicide to non-psychiatric controls. BDNF levels are altered in depression and other psychiatric disorders. In addition, most suicides

occur in the context of a psychiatric disorder, suggesting that individuals with a psychiatric illness are a particularly vulnerable population for suicidal behaviour. In order to determine the association between BDNF and suicidal behaviour, a comparison group should be derived from a psychiatric population, in addition to healthy controls. Maheu et al.'s study was the only postmortem study that did not find a significant association between BDNF and suicide. The differences found in the other three studies could have resulted from altered BDNF levels associated with psychiatric disorders rather than suicidal behaviour. Therefore, one should be cautious when interpreting the results of the other studies, as their significant findings may not represent an association between BDNF and suicide.

Another important factor to consider is the effect of psychotropic medications on BDNF levels. Only one study, Karege et al. (28), explored this variable. They separated the group of people who died by suicide by toxicology by determining the presence of therapeutic drugs in the body. They found differences in BDNF levels among the groups. They found a significant association between BDNF level and suicidal behaviour when comparing drug-free suicide completers to controls, but not when comparing drug-treated suicide completers to controls. Future studies should investigate and control for the effects of antidepressants and other medications on BDNF levels in postmortem suicide deaths.

Postmortem studies are subject to a number of limitations, making it difficult to draw sound conclusions from them. Factors prior to death can affect the integrity of the brain's morphology and biochemical content (46). Depending on the cause and manner of death, changes in blood oxygenation, brain perfusion, and acid-base balance can have

varying effects on the brain and on the variables of interest in postmortem studies. Different methods of suicide can produce different effects on the brains. Postmortem interval (PMI), the time between death and freezing or fixing of the brain tissue, also influences the quality of the tissue. PMI can have complex and unknown effects on the outcome measure being studied (46). Only two of the postmortem studies of BDNF and suicide adjusted for confounding variables in their analyses (28, 40). Both Maheu et al. and Karege et al. adjusted for PMI and age, and Maheu et al. also adjusted for brain pH. Future postmortem studies should assess and control for factors that influence the integrity of the brain tissue samples.

Bearing in mind these limitations, one can cautiously conclude from the existing evidence that an association may exist between brain levels of BDNF (particularly in the hippocampus and prefrontal cortex) and completed suicide. However, additional studies with larger samples and psychiatric comparators are needed to confirm this association.

3.5.2 Cerebrospinal fluid BDNF levels and attempted suicide

The one study of CSF levels of BDNF and suicidal behaviour, by Martinez et al. (41), found that increased levels of BDNF were significantly associated with higher levels of suicidal ideation. This finding is contradictory to the hypothesis that lower levels of BDNF are associated with suicidal behaviour. However, the sample size for the analysis was very small (12 participants) and the analysis did not adjust for confounding factors. Additional well-powered studies are necessary to explore this association. At this point, no conclusions can be drawn regarding the association of CSF levels of BDNF and suicidal behaviour.

3.5.3 Serum BDNF levels and attempted suicide

The six studies of serum BDNF levels and suicidal behaviour vary widely in their findings. Of the studies that looked at attempted suicide, three found significant associations and three did not. Two of the studies also investigated suicidal ideation and found a significant relationship with BDNF levels.

The limitations of the studies' methodologies could have resulted in biased estimates and inconsistent findings. The sample sizes were generally modest, with case groups ranging from 10 to 31 participants. None of the studies adjusted for confounding variables in their analyses, even though observational studies are inherently prone to influences by many confounding variables. Of the six studies of serum BDNF and suicidal behaviour, only two performed adjusted analyses. Grah et al. (37) adjusted for age, sex, and therapy, while Pinheiro et al. (38) adjusted for previous psychiatric treatment and stressful life events during pregnancy.

Another factor that could account for the inconsistent findings among studies is the variation in time periods between suicide attempts and BDNF measurement. While Deveci et al.'s study included individuals who were hospitalized for a recent suicide attempt, other studies included individuals with a lifetime history of suicide attempts. In studies including participants with a lifetime history of suicide attempts, the BDNF measurement could have occurred within weeks, months, or years of the suicide attempt, and the precise time interval is neither known nor accounted for in the analysis. Because BDNF levels vary over time in response to a number of external factors, the BDNF measurements in these studies may not represent the levels at the time of the suicide attempts. While it is unclear whether BDNF levels constitute a predisposing or

precipitating risk factor for suicidal behaviour, studies should take into consideration the time intervals between attempt and BDNF measurement and aim for consistency. It is likely that associations between BDNF levels and suicidal behaviour will vary depending on when BDNF levels are assessed. Because only one of the six studies of BDNF level and attempted suicide included recent cases, no conclusions can be drawn regarding the relative strength of the association in recent as opposed to past suicide cases. Future studies should aim to measure BDNF in closer proximity to the suicide attempt in order to minimize the effects of unmeasured confounders that may be influenced by differences in time.

An additional point to consider is the varying methods of sample selection among studies. While some of the case-control studies separately recruited individuals who had made suicide attempts and compared them to non-suicidal controls (29, 34), other studies recruited individuals from a psychiatric population and retrospectively assessed their history of suicide attempts (37, 43). Future studies should aim to separately recruit individuals who had attempted suicide and non-suicidal psychiatric controls in order to attain larger samples of individuals with suicide attempts and to increase the generalizability of the findings beyond individuals with a specific psychiatric disorder.

The meta-analysis of case-control studies of serum BDNF in individuals with suicide attempts and psychiatric controls revealed a small effect size of –0.32. The p-value was not significant (p=0.36). The high heterogeneity associated with this pooled estimate could be attributed to the diversity in the sample populations. Liang et al.'s sample consisted of patients with major depression, Huang and Lee's sample consisted of patients with schizophrenia, and Deveci et al.'s sample consisted of individuals with

suicide attempts with no major psychiatric disorder and control participants with major depression. This meta-analysis may be underpowered due to the small number of studies included and the low sample sizes in each study. Nonetheless, this is an important finding, as it suggests that individuals who attempt suicide do not have significantly altered serum BDNF levels compared to psychiatric controls.

Further research is necessary to elucidate the relationship between serum BDNF levels and suicidal behaviour, and to ascertain whether the relationship depends on the timing of measurements. Consistent definitions of suicidal behaviour, research methodology, and adjustment for important confounding factors (such as medication use, body mass index, and smoking status (47, 48, 18)) may help to produce a clearer understanding of the relationship. Currently, the evidence does not provide convincing support for an independent association between serum BDNF levels and suicidality.

3.5.4 Plasma BDNF levels and attempted suicide

The three studies of plasma BDNF levels and suicidal behaviour present conflicting evidence of the relationship. Two of the three studies (30, 31) found significant associations between plasma BDNF levels and attempted suicide, while the third (45) did not. It is interesting to note that two studies with very similar study designs (30, 45), in which patients with depression who were hospitalized for recent suicide attempts were compared to hospitalized non-suicidal patients with depression and healthy controls, had opposing findings. Kim et al.'s 2007 study found significantly low BDNF levels in suicidal individuals compared to both control groups, but Lee and Kim's study in 2009 found no relationship between BDNF and suicidal behaviour. The inconsistency in findings could be due to a number of factors. In all three of these studies univariate

analyses were used to compare BDNF levels among groups. While participants were matched on some variables (age and sex), no variables were adjusted for in the analyses. In addition, the sample sizes of these three studies are small; the group of individuals with suicide attempts varied from 20 to 32 individuals. Future studies should be conducted using larger samples, and using statistical analyses that adjust for confounding variables such as medication use, body mass index, and smoking status (47, 48, 18).

Another consideration is that, like in the studies of serum BDNF levels, these studies vary in the time periods between BDNF measurement and suicide attempt. Both Kim et al.'s and Lee and Kim's studies included individuals hospitalized for recent suicide attempts, while Lee's study included individuals with a lifetime history of suicide attempts. However, this does not explain the differences in findings, since the inclusion of recent versus past suicide cases did not determine whether a significant association was found between BDNF level and suicidal behaviour.

Seeing that these three studies were all conducted at a single research centre in Korea, and may not have included independent samples, additional studies conducted in other locations with diverse sample populations will contribute valuably to the literature.

As of yet, the studies of plasma BDNF levels and suicidal behaviour are few in number, inconsistent in their findings, and subject to methodological limitations. No conclusions can be drawn from the existing evidence on the association between plasma levels of BDNF and attempted suicide.

3.5.5 GRADE quality of evidence

While the protocol for this systematic review stated that the Grading of Recommendations, Assessment, and Evaluation (GRADE) framework would be used to

report the quality of evidence, it was deemed unnecessary to do so. The GRADE framework provides a systematic approach to consider and report risk of bias, imprecision, inconsistency, indirectness of study results, and publication bias. The GRADE framework is used to summarize and evaluate the evidence according to outcome, and is useful when the results of the studies have been combined statistically. Seeing as only 3 of the 14 included studies were pooled in a meta-analysis, it was not possible to evaluate the quality of the evidence using this framework. Furthermore, the GRADE framework is best suited to summaries of randomized controlled trials and is rarely used for observational studies such as these.

3.6 CONCLUSIONS

This is the first systematic review to explore associations between BDNF levels and suicidal behaviour. The meta-analysis of studies examining serum BDNF levels and attempted suicide revealed no significant association. The qualitative review of the literature revealed that the current evidence does not provide consistent support for an association between BDNF and suicidal behaviour. The findings of this systematic review are not in accordance with the hypothesis that lower levels of BDNF are linked to suicidal behaviour. It is possible than an association exists in parts of the brain and bloodstream, but the studies vary substantially in their methods and results, making it difficult to draw sound conclusions. The studies are also subject to a number of methodological limitations. As of yet, the studies conducted are few in number and have high risk of bias. Moreover, distinguishing the role of BDNF in suicidal behaviour from its role in mental illness is a key difficulty across studies. As this is a relatively new area of research, currently the evidence does not warrant using measures of BDNF in a

clinical setting to assess suicide risk. Further studies that are well-powered, include psychiatric comparator groups, and adjust for important confounders will help to elucidate this relationship and may provide valuable information to clinicians and researchers.

3.7 ACKNOWLEDGEMENTS, COMPETING INTERESTS, AND AUTHOR CONTRIBUTIONS

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The authors declare that they have no competing interests.

RE conceived and designed the study, interpreted and analyzed the data, and wrote and revised the manuscript. SP participated in the methodology and manuscript writing. LB participated in the development of the search strategy. RA and LM critically revised the manuscript. ZS conceived and designed the study, participated in the methodology, and critically revised the manuscript. All authors read and approved the final manuscript.

3.8 FIGURES AND TABLES

Figure 3.4.1: PRISMA Flow Diagram

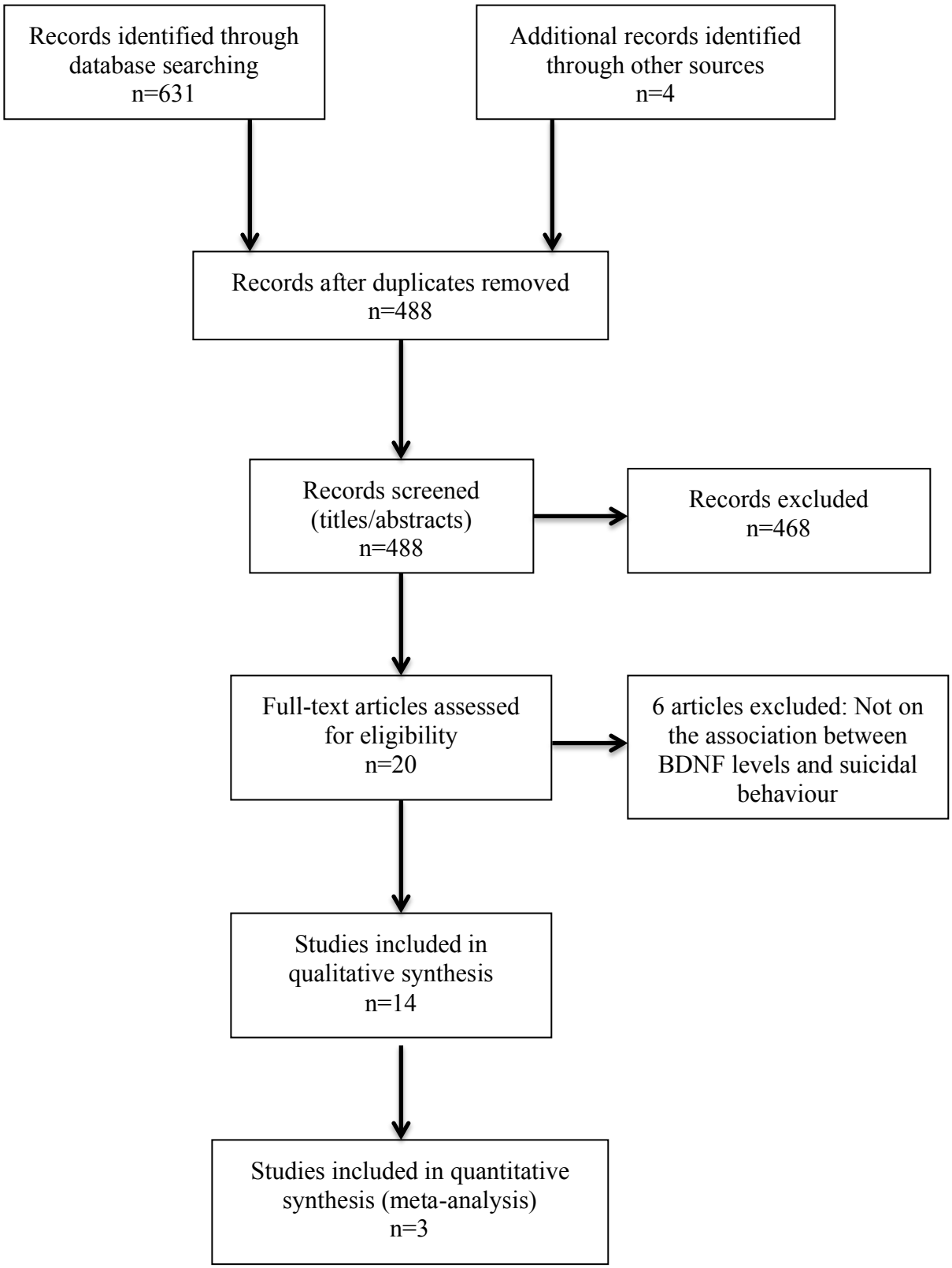

Figure 3.4.6: Forest Plot of Mean Differences in Serum BDNF in Suicide Attempters and Psychiatric Controls

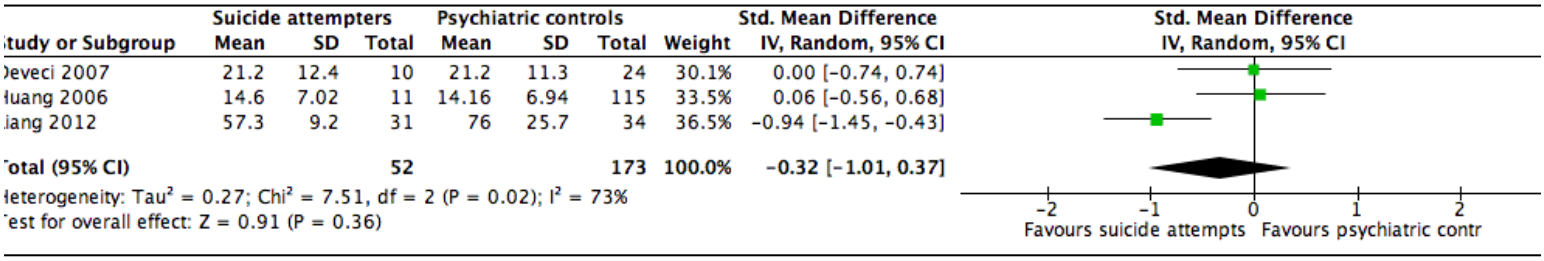

Table 3.3.1: Medline Search Strategy

MEDLINE (n=124)	1. exp Suicide/ 2. suicid*.mp. 3. exp Self-Injurious Behavior/ 4. (self harm* or self inflict* or self injur* or self wound* or self mutilat* or self poison* or overdose).mp. 5. automutilat*.mp 6. 1 or 2 or 3 or 4 or 5 7. brain derived neurotrophic factor.mp. or Brain-Derived Neurotrophic Factor/ 8. bdnf.mp 9. 7 or 8 10. 6 and 9

Table 3.4.2: Study Characteristics

Author (year)	Study design and sample groups	Participants (n)	Definition of suicidal behaviour	Type of BDNF measurement (units)	Mean (SD) BDNF levels	P-value (difference between groups)	Variables adjusted for
Postmortem Brain Studies							
Banerjee et al. (2013)	Case-control Cases of suicide vs. non-psychiatric healthy controls	Suicide: 21 Control: 19	Completed suicide	Brain – hippocampus (pg/ml)	Suicide: 19.5[a] Control: 44[a]	<0.001	None
Dwivedi et al. (2003)	Case-control Cases of suicide vs. non-psychiatric controls	Suicide: 27 Control: 21	Completed suicide	Brain – PFC, hippocampus (optical density)	**PFC** Suicide + MDD: 0.94 (0.22) Suicide + other psychiatric disorder: 0.88 (0.28) Control: 1.61 (0.39) **Hippocampus** Suicide+MDD: 1.04 (0.20) Suicide+other psychiatric disorder: 1.03(0.22) Control: 1.71 (0.44)	<0.001 <0.001	None
Karege et al. (2005)	Case-control Cases of suicide vs. non-psychiatric controls	Suicide: 30 Control: 24	Completed suicide	Brain – PFC, hippocampus, entorhinal cortex (ng/g)	**Hippocampus** Drug-free MDD: 17.7 (2.9) Drug-free others: 16.8 (3.1) Drug-treated MDD: 23.3 (2.2) Drug-free controls: 24.5 (3.6) **PFC** Drug-free MDD: 13.8 (2.6) Drug-free others: 12.7 (2.6) Drug-treated MDD: 17.9 (2.9) Drug-free controls: 17.5	<0.001 <0.002	PMI, age

					(3.0) **Entorhinal cortex** Drug-free MDD: 14.1 (2.1) Drug-free others: 13.4 (2.4) Drug-treated MDD: 12.9 (2.3) Drug-free controls: 13.4 (2.5)	Not significant	
Maheu et al. (2013)	Case-control Depressed individuals (divided into suicide victims vs. non-suicide deaths) vs. non-psychiatric controls	Depressed suicide (12) Depressed non-suicide (10) Control (14)	Completed suicide	Brain – BLA, CeA (optical density)	**BLA** Depressed suicide: 1.19 (0.80) Depressed non-suicide: 1.67 (1.42) Control: 1.08 (0.34) **CeA** Depressed suicide: 0.95 (0.20) Depressed non-suicide: 1.87 (1.30) Control: 0.97 (0.16)	Not significant Not significant	PMI, brain pH, age
CSF Studies							
Martinez et al. (2012)	Case-control Depressed patients vs. non-depressed patients	Depressed (18) Non-depressed (25)	Suicidal ideation	CSF (pg/ml)	NA	Correlation between BDNF concentration and SSI score: r=.62, P=.033, n=12	None
Serum BDNF Studies							
Deveci et al. (2007)	Case-control Suicide attempters vs. non-suicidal MDD patients vs. healthy controls	Suicide (10) Non-suicidal MDD (24) Healthy controls (26)	Suicide attempt	Serum (ng/ml)	Suicide: 21.2 (12.24) Non-suicidal MDD: 21.2 (11.3) Control: 31.4 (8.8)	Suicide vs. healthy controls: 0.004 Suicide vs. MDD: not significant	None
Grah et al. (2014)	Case-control MDD patients vs. PD patients vs. AD patients vs. healthy controls	Suicide (96) Control (106) [b]	Suicide attempt	Serum (ng/ml)	**RDD** [c] Suicide: 11.8 (8.88–14.73) Control: 12.8 (10.83–14.78) **PD** Suicide: 10.7 (7.04–14.26) Control: 15.7	0.07 0.003	Age, sex, therapy

					(10.29–21.02) **AD** Suicide: 12.6 (9.63–15.58) Control: 15.4 (12.04–18.77)	0.009	
Huang & Lee (2006)	Case-control Schizophrenic patients vs. healthy controls	Suicidal schizophrenic (11) Non-suicidal schizophrenic (115) Healthy control (96)	Suicide attempt	Serum (ng/ml)	Suicidal schizophrenic: 14.60 (7.02) Non-suicidal schizophrenic: 14.16 (6.94)	0.841	None
Liang et al. (2012)	Case-control Depressed patients (with or without suicide attempt) vs. healthy controls	Suicidal depressed (31) Non-suicidal depressed (34) Healthy control (30)	Suicide attempt, suicidal ideation	Serum (ng/ml)	Suicidal depressed: 57.3 (9.2) Non-suicidal depressed: 76.0 (25.7) Healthy control: 113.8 (44.4)	Suicide attempt: <0.01 Suicidal ideation: <0.01	None
Park et al. (2014)	Cross-sectional MDD patients	Suicidal MDD (18) Non-suicidal MDD (33)	Suicide attempt	Serum (ng/ml)	Suicidal MDD: 21.93 (24.71) Non-suicidal MDD: 24.71 (7.7)	0.3	None
Pinheiro et al. (2014)	Cross-sectional Postpartum women	History of suicide attempts (12) No suicide history (178)	Suicide attempt, suicidal ideation	Serum (ng/ml)	Suicide: 2.11 (1.42) Control: 2.37 (1.26)	0.6 Linear regression of PPAD and suicide risk: -0.912 (-1.73– -0.09) p=0.029	Previous psychiatric treatment, stressful life events during pregnancy
Plasma BDNF Studies							
Kim et al. (2007)	Case-control Suicidal depressed patients vs. non-suicidal depressed patients vs. healthy controls	Suicidal depressed (32) Non-suicidal depressed (32) Control (30)	Suicide attempt	Plasma (pg/ml)	Suicidal depressed: 430.5 (397.0) Non-suicidal depressed: 875.80 (663.02) Control: 889.4 (611.3)	0.002	None
Lee et al. (2007)	Case-control Depressed patients vs. healthy controls	Suicidal depressed (28) Non-suicidal depressed (49) Control (95)	Suicide attempt	Plasma (pg/ml)	Suicidal depressed: 386.61 (362.39) Non-suicidal depressed: 689.66 (404.65) Control: 819.20 (347.05)	<0.001	None

Lee & Kim (2009)	Case-control Suicidal depressed patients vs. non-suicidal depressed patients vs. healthy controls	Suicidal depressed (20) Non-suicidal depressed (20) Control (20)	Suicide attempt	Plasma [d] (pg/ml)	Suicidal depressed: 713.04 (236.56) Non-suicidal depressed: 693.98 (347.84) Control: 709.05 (172.12)	0.971	None

[a] Mean BDNF values estimated from inspection of graph.
[b] 3 diagnosis groups: Recurrent depressive disorder, personality disorder, adjustment disorder.
[c] Medians and interquartile ranges presented instead of means and SD.
[d] BDNF was measured in 3 types of sample: platelet-rich plasma, platelet-poor plasma, and platelets. Results are presented here for only the platelet-poor plasma measurement.

Abbreviations: BDNF, brain-derived neurotrophic factor; SD, standard deviation; MDD, major depressive disorder; PFC, prefrontal cortex; PMI, postmortem interval; BLA, basolateral amygdala; CeA, central nucleus of the amygdala; RDD, recurrent depressive disorder; PD, personality disorder; AD, adjustment disorder; PPAD, postpartum affective disorder; CSF, cerebrospinal fluid

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CHAPTER 4

Study 2

Exploring the Association between Serum BDNF and Attempted Suicide

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Keywords: BDNF, suicidal behaviour, risk factors

4.1 ABSTRACT

Suicide is a leading cause of death and a significant public health concern. Brain-derived neurotrophic factor (BDNF), a protein important to nervous system function, has been implicated in psychiatric disorders and suicidal behaviour. We investigated the association between serum levels of BDNF and attempted suicide in a sample of 281 participants using a case-control study design. Participants were recruited from clinical and community settings between March 2011 and November 2014. Cases (individuals who had attempted suicide) (n=84) were matched on sex and age (within five years) to both psychiatric controls (n=104) and community controls (n=93) with no history of suicide attempts. We collected fasting blood samples, socio-demographic information, physical measurements, and detailed descriptions of suicide attempts. We used linear regression analysis to determine the association between BDNF level (dependent variable) and attempted suicide (key exposure variable), adjusting for age, sex, body mass index, current smoking status, and antidepressant use. 250 participants were included in this analysis. In the linear regression model, attempted suicide was not significantly associated with BDNF level (β= 0.28, SE=1.20, P=0.82). Our findings suggest that no significant association exists between attempted suicide and BDNF level. However, the findings need to be replicated in a larger cohort study.

4.2 INTRODUCTION

Suicide claims nearly one million lives each year, making it a leading cause of death worldwide and a significant public health concern (1). The devastating effects of suicide are felt at the family, community, and societal levels. Suicidal thoughts, plans, and acts intended to end one's life all comprise the complex phenomenon of suicidal behaviour. Non-fatal suicidal behaviours are 10-20 times more common than completed suicide (2). Attempted suicide is also an important risk factor for future completed suicide (1).

Many risk factors are thought to contribute to the risk of suicidal behaviour. These include biological, psychological, social, and environmental factors (3-5). Psychiatric disorders are highly predictive of suicidal behaviour, among which mood disorders pose significant risk (5). Population level estimates suggest that 90% of attempted and completed suicides occur in the context of a psychiatric disorder (3). Other known risk factors include chronic illness, substance use disorders, and demographic variables such as age and sex (3). These biological and psychological factors point to a predisposition toward suicidal behaviour in some individuals. However, these factors alone do not predict suicidal behaviour. Social and environmental risk factors also play a role, and include unemployment, low educational attainment, unmarried status, and a lack of social support (3). Incidents of suicidal behaviour likely result from the interaction between biological and psychosocial factors.

Brain-derived neurotrophic factor (BDNF) is the most abundant member of the neurotrophins, a family of proteins that regulate the survival, development, maintenance, and function of vertebral nervous systems (6). BDNF is involved in many neural

processes including neurogenesis, nerve growth, neuroplasticity, and neurotransmission (7). Altered levels of BDNF have been associated with several psychiatric conditions. Low blood levels of BDNF have been linked to depression (8, 9) and reduced BDNF expression in the brain has been linked to stress (10, 11). Both depression and stress are major risk factors for suicidal behaviour (7). Since BDNF is intrinsic to optimal nervous system function, pathological changes in BDNF levels are a possible cause of neurobiological deficits that impair one's ability to adapt to difficult situations (7).

Recent research has examined the association between BDNF and suicidal behaviour (12-23). The literature on this topic has been summarized and evaluated in a systematic review and meta-analysis by Eisen et al. (24). Some studies have investigated postmortem levels of BDNF in the brains of suicide victims (12-14), while other studies have measured peripheral BDNF levels in clinical samples (15-23). Postmortem studies have found significantly lower BDNF levels in the hippocampus and prefrontal cortex in individuals who died by suicide compared to individuals who died of other causes (12-14). Studies comparing peripheral levels of BDNF in individuals with and without a history of suicidal behaviour have shown conflicting results (15-20). Some studies of serum levels of BDNF have shown significantly reduced levels in individuals with suicide attempts compared to both psychiatric and healthy controls (15, 16). However, other studies making the same comparison found no significant relationship (17-20). Studies of plasma BDNF levels in individuals with depression with and without a history of suicidal behaviour are similarly conflicted in their findings (21-23).

Studies of the association between BDNF levels and suicidal behaviour are few in number and limited in methodology. The sample sizes are generally small, with some

comparison groups containing as few as 10 participants (for examples, see (17, 18)). As well, nearly all previous studies conducted univariate analyses to compare BDNF levels among patient groups, and few studies adjusted for confounding variables in their analyses. Furthermore, many previous studies compare BDNF levels between groups of individuals with and without a lifetime history of attempted suicide (for examples, see (15, 18, 22)). In these studies, the BDNF measurements may not represent BDNF levels at the time of the attempt. Additional research is required to establish the relationship between BDNF levels and suicidal behaviour.

In the present study we examine the relationship between serum BDNF levels and recent suicide attempts (within the past three months) in a large clinical sample using a case-control study design.

4.3 METHODS

4.3.1 Data collection

The data used in this study were collected from the Study of Determinants of Suicide Conventional and Emergent Risk (DISCOVER), an observational matched case control study that aims to understand the risk factors involved in suicidal behaviour (36). Cases (participants hospitalized for a suicide attempt) were matched on age and sex to two control groups (psychiatric patients and community controls). Data were collected at St. Joseph's Healthcare Hamilton, Hamilton Health Sciences Hospitals, and the Hamilton City community, in Ontario, Canada. Data collection began in March 2011 and ended in November 2014. The study was approved by the Hamilton Integrated Research Ethics Board (HiREB) (number 10-661 for St. Joseph's Healthcare Hamilton and 11-3479 for Hamilton Health Sciences Hospitals). The methods of this study were performed in

accordance with the HiREB guidelines. This study follows the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines (37).

4.3.2 Inclusion and exclusion criteria

The study included men and women 18 years and older who were able to provide written informed consent, communicate in English, and follow study procedures. Cases were defined as individuals who made a serious suicide attempt (defined as self-directed injury with intent to die) within in the last three months, were admitted to hospital, and required medical or psychiatric intervention. Cases were matched based on sex and age (within five years) to both psychiatric and community control groups. The psychiatric control group consisted of individuals with psychiatric disorders with no history of suicide attempts, who were admitted to hospital within the same time frame as the cases. The community control group consisted of individuals with no history of suicide attempts who were recruited from community and non-psychiatric clinical areas. Participants were excluded if they were unable to provide informed consent or follow study procedures.

4.3.3 Recruitment

Upon recruitment, fasting blood and urine samples were collected and a structured interview was conducted. Data were obtained on socio-demographic variables (age, sex, ethnicity, religion, marital status, education, employment, and social support), medical history and current medications, psychopathology, physical measurements, and suicidal behaviour. The study questionnaires were compiled using previously validated diagnostic and assessment tools including the Mini-International Neuropsychiatric Interview (38) and the Beck Suicide Intent Scale (39). For participants in the case group, a detailed

description of the suicide attempt was recorded. All assessments were administered in hospital or community by trained research staff.

4.3.4 Laboratory analysis

12-hour fasting blood samples were collected, processed, and stored at the Clinical Research and Clinical Trials Laboratory at Hamilton General Hospital. Samples were processed within two hours of collection. After 30 minutes of clotting time, samples were spun at 1500xg (3000 rpm) for 15 minutes until blood was well separated. Samples were then aliquotted into 2 mL cryovials and stored in liquid nitrogen for future analyses. Serum BDNF level was assayed using Quantikine® ELISA Human BDNF Immunoassay (R&D Systems Inc.). All analyses were conducted blindly according to standard procedures.

4.3.5 Statistical analyses

We used descriptive statistics to summarize baseline characteristics of the sample. Means and standard deviations (SD) were reported for continuous variables, and counts and percentages were reported for categorical variables. Analysis of variance tests (ANOVA) were used to compare means for continuous variables and chi-square tests were used to compare proportions for categorical variables.

We employed a linear regression to model the association between attempted suicide and serum BDNF level. The confounders included in the model were selected a priori based on previous literature (age, sex, smoking status, body mass index, and antidepressant use). Serum BDNF level was the outcome variable, and case/control group (suicide attempt, psychiatric control, or community control) was the independent variable of interest. We chose to perform a linear regression analysis with BDNF level as the

dependent variable because, while it is unknown whether BDNF is related to suicidal behaviour in a causal manner, it is known that many factors can influence BDNF levels. This analysis allowed us to investigate the relationship between attempted suicide and BDNF level in the context of other factors known to affect BDNF level.

We performed a sensitivity analysis using linear regression to explore the association between attempted suicide and serum BDNF level by comparing the case group to each control group individually. The same confounders listed above were included in the sensitivity analyses models. All analyses were performed using R version 3.0.2 (40).

4.4 RESULTS

A total of 281 participants were recruited, including 84 cases (individuals who had attempted suicide), 104 psychiatric controls, and 93 community controls (see Figure 4.4a for flow diagram of recruitment). The mean age of the sample was 43.7 years (standard deviation [SD]=14.6, range: 18-73). The sample contained approximate equal numbers of males and female participants (52.2% female). The three groups differed significantly on a number of demographic variables including education level and employment status, with the case group containing the fewest individuals with secondary education and current employment. Several psychiatric disorders were most common in the case group, including major depressive disorder, anxiety disorders, alcohol and substance abuse, and antisocial personality disorder. The mean BDNF level was 24.21 ng/ml (SD=7.19) in the case group, 23.88 ng/ml (SD=6.95) in the psychiatric control group, and 24.77 ng/ml (SD=7.01) in the community control group (see Figure 4.4b). The sample characteristics are summarized in Table 4.4.

Mean BDNF levels for the largest psychiatric disorder categories (mood disorders and anxiety disorders) were compared using ANOVA. In both categories, no significant differences were found in mean BDNF level among cases, psychiatric controls, and community controls (F=0.55, P=0.58 for mood disorder; F=0.20, P=0.82 for anxiety disorder).

4.4.1 Primary analysis

The linear regression analysis did not demonstrate a significant association between attempted suicide and BDNF level (β= 0.28, standard error [SE]=1.20, P=0.82). Of the covariates included (age, sex, smoking status, body mass index, and antidepressant use), only antidepressant use was significantly associated with BDNF level (β=-2.50, SE=0.99, P=0.012). The analysis included 250 out of 281 participants (11% missing data). The assumptions of linearity, independence, normality, and homoscedasticity were tested and all were satisfied. The variance inflation factors (VIF) for all variables were below 1, indicating that multicollinearity was not a concern. See Table 4.4.1 for linear regression model.

When this analysis was repeated with the inclusion of mood disorders and anxiety disorders as covariates, neither was significantly associated with BDNF level β=-0.79, P=0.54 for mood disorder; β=2.20, P=0.07 for anxiety disorder). The overall results were the same as those of the primary analysis.

4.4.2 Sensitivity analyses

Linear regression analyses were conducted to examine the relationship between attempted suicide and BDNF level when cases are compared to each control group individually. In the first model cases were compared to psychiatric controls, and in the

second model cases were compared to community controls. In both models, attempted suicide was not significantly associated with BDNF level (β=0.85, SE= 1.13, P=0.46 in the first model; β=0.20, SE=1.27, P=0.87 in the second model).

4.5 DISCUSSION

Upon examining the association between serum BDNF level and attempted suicide using a case-control design, our findings demonstrate that serum BDNF level was not significantly associated with attempted suicide. The association was true regardless of comparison group.

Our study's findings are not in accordance with the hypothesis that lower levels of BDNF are associated with suicidal behaviour. Our findings conflict with previous literature on this relationship, including a study by Dawood et al. that examined the association between internal jugular venous BDNF and suicidal behaviour (25). Dawood et al. found a negative correlation between suicide risk and BDNF concentration, which supports the notion that reduced brain levels of BDNF are involved in the pathogenesis of depression and suicide. However, the study's small sample size (16 participants) and unadjusted statistical analyses may have resulted in a biased estimate.

Only six previous studies have compared serum BDNF levels in groups with and without suicidal behaviour. While two of these studies found a significant association between serum BDNF and attempted suicide (15, 16), the remaining studies found no significant association (17-20). While our study's findings are not in accordance with the hypothesis that lower levels of BDNF are associated with depression and suicidal behaviour, they are consistent with other studies of this relationship.

This study has a number of strengths that distinguish it from other studies in this area. Our sample of 281 participants was larger than most previous studies, some of which included only 40 participants in total. As well, since our case group comprised individuals with recent suicide attempts (within three months of recruitment), the BDNF measurement was taken within a consistently short period of time relative to the attempt. Other studies have included participants with a lifetime history of suicide attempts. In these studies, the BDNF measurements may not represent BDNF levels near the time of the attempt. An additional strength of this study is the inclusion of two control groups, a psychiatric control group and a community control group. Since the majority of suicide attempts occur in the context of a psychiatric disorder (3), it is important to include both comparisons in studies of suicidal behaviour. Finally, unlike most previous studies, we performed adjusted analyses to examine the relationship between BDNF level and attempted suicide. We adjusted for a number of variables known to be associated with altered BDNF level. The results of other studies may have been influenced by confounding variables such as age, smoking status, and body mass index.

Comparing BDNF levels among the three groups (cases, psychiatric controls, and community controls) within categories of psychiatric disorders (mood disorders and anxiety disorders) revealed that mean BDNF levels did not differ significantly within either the mood disorder or anxiety disorder categories. As well, when each of these diagnosis categories was include in the linear regression model, neither was significantly associated with BDNF level. While there is evidence to suggest that BDNF levels are related to several psychiatric disorders, including depression (8, 9), bipolar disorder (26, 27), schizophrenia (28), and substance use disorder (29, 30), as of yet, it is unknown

whether the relationship between BDNF and suicidal behaviour depends on the presence of an underlying psychiatric disorder. Future research should explore this topic.

Our finding that antidepressant use was associated with decreased BDNF level is contradictory to most previous evidence. In a meta-analysis conducted by Molendijk et al. in 2013, serum BDNF levels were found to be increased in antidepressant-treated patients with depression compared to untreated patients with depression (31). The meta-analysis included 28 comparisons, of which only four found the opposite effect, with increased BDNF levels in untreated groups with depression (32-35). These studies generally concluded that the effects of antidepressants on BDNF concentrations depends on the type of antidepressant used, and even within the same category of antidepressant (selective serotonin reuptake inhibitors, for instance), different antidepressants vary in their effects on BDNF level. Since all types of antidepressants were combined into one variable in our analysis, this could explain our unexpected result. Molendijk et al.'s meta-analysis also concluded that most studies of BDNF levels in antidepressant-treated and untreated individuals with depression are underpowered, and when publication bias is accounted for, the effect size is smaller than previously thought (31). This puts into question the notion that high BDNF levels are in fact associated with antidepressant use. Another important point to consider is that of the 80 individuals in our sample who were taking antidepressants, 41% did not meet the requirements for major depressive disorder according to the Mini-International Neuropsychiatric Interview. These individuals could have been taking antidepressant medications for various reasons, including treatment of anxiety, chronic pain, or insomnia. This may partly explain our finding, since other

factors may be responsible for the low BDNF levels in patients on antidepressants in our sample.

Future studies should aim to replicate these findings using a cohort study design. Additional research should also explore other potential biomarkers for suicidal behaviour. Since there is evidence for a connection between hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis and suicidal behaviour (2), potential biomarkers could include cortisol or pro-inflammatory cytokines.

4.6 CONCLUSION

Our case-control study shows no significant association between serum BDNF level and attempted suicide. However, the finding will need to be replicated in a larger cohort study.

4.7 ACKNOWLEDGEMENTS, COMPETING INTERESTS, AND AUTHOR CONTRIBUTIONS

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The authors declare that they have no competing interests.

RE developed the research question, analyzed and interpreted the data, and wrote and critically revised the manuscript. SP contributed to data analysis and interpretation, and critically revised the manuscript. MB and BD assisted with recruitment and critically revised the manuscript. WE was the research assistant for this study and was responsible for recruitment, interviews, and data collection. JD was the study coordinator responsible for the daily running of the study, training research assistants and study personnel on study-related procedures. SR was the study project manager responsible for the study conduct; with ZS she designed the study protocol, provided supervision of study personnel, and coordinated the overall study management. JV, HS, NH, and EI are nurses specializing in psychiatry; they recruited hospitalized study patients, conducted interviews, collected blood samples, and provided feedback on participants' recruitment and study procedures. PM was responsible for database management, quality and edits, checks of data, and provision of reports, including age and sex matching status, to the study team for the weekly study meetings. SI was responsible for data management and preparation of data reports to the team. MD designed the dietary tool and analyzed the dietary data for the DISCOVER study. JB provided advice on study procedures including

recruitment and the inclusion of psychiatric controls based on her expertise in emergency psychiatry and suicide prevention. RA critically revised the manuscript. LM contributed to data analysis and interpretation, and critically revised the manuscript. LT was responsible for the case-control study design, and overall methodological and statistical aspects of the study. ZS conceived the study and was principally responsible for the conduct of the study and obtaining funding. ZS also developed the research question, contributed to data analysis and interpretation, and critically revised the manuscript. All authors have reviewed and approved the final manuscript.

4.8 FIGURES AND TABLES

Figure 4.4a: Flow diagram for recruitment of participants

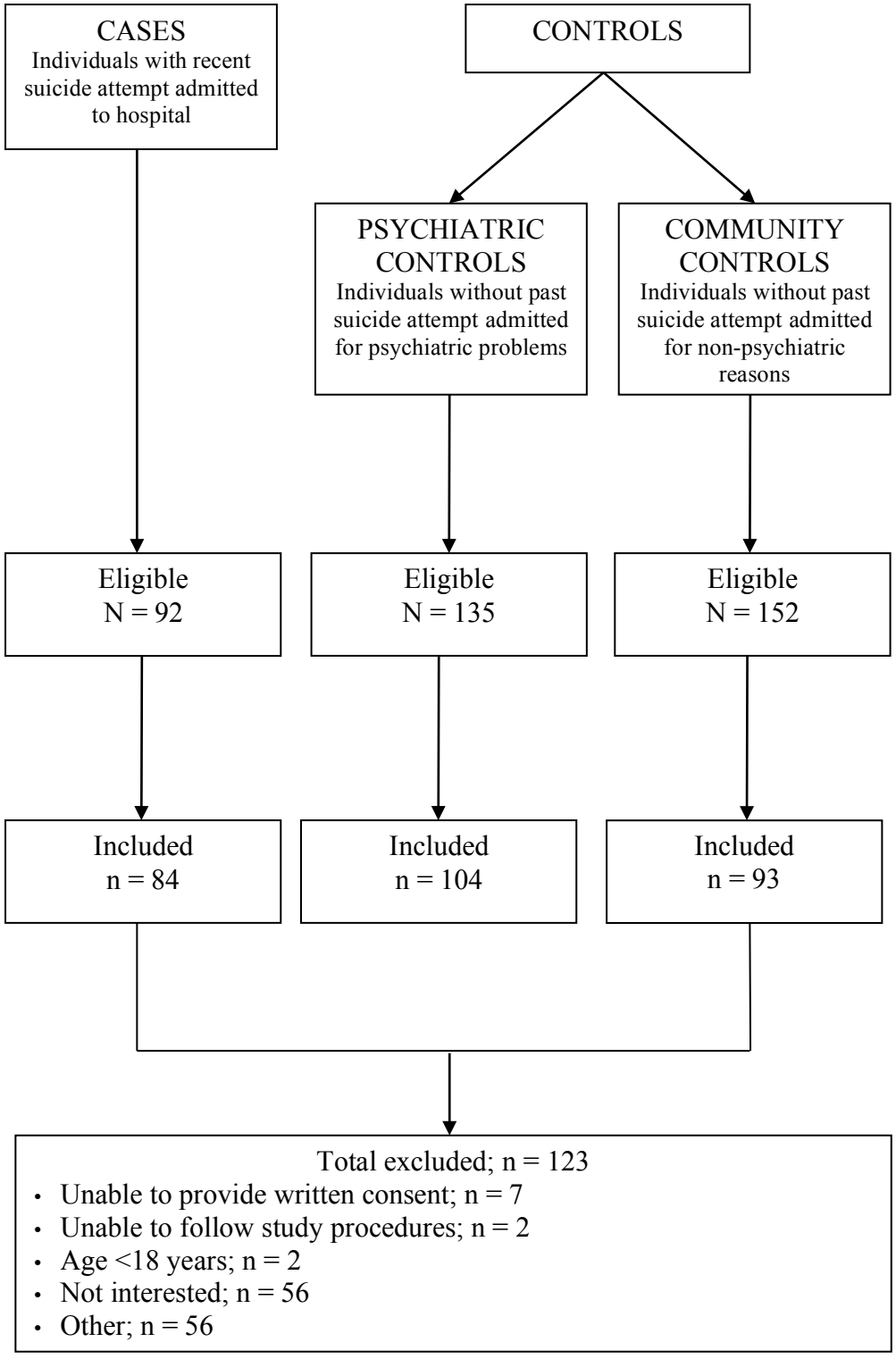

Figure 4.4b: Serum BDNF Level by Group

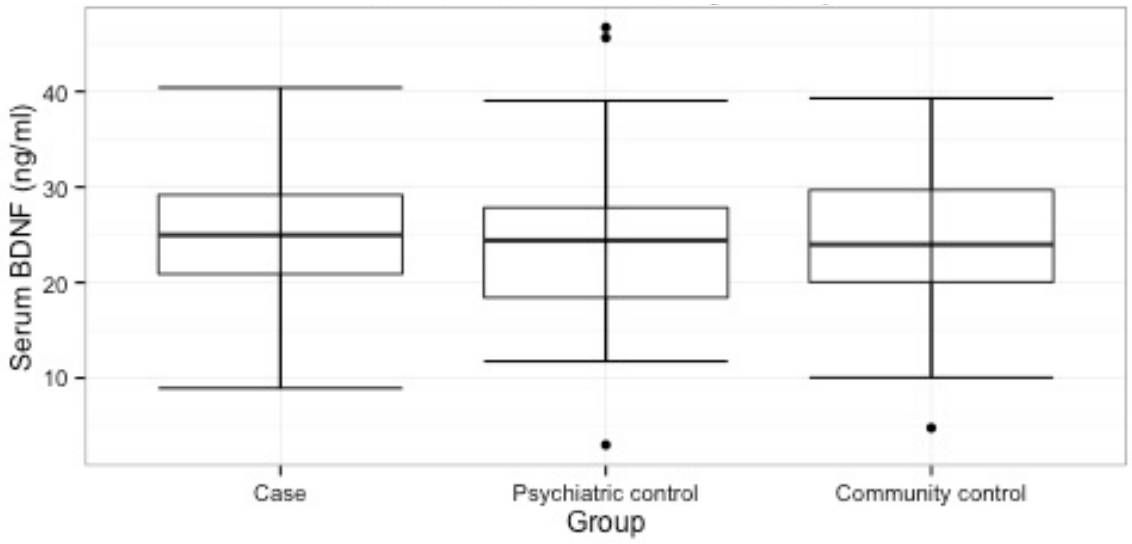

Table 4.4: Baseline Sample Characteristics

	Cases (n=84)	Psychiatric controls (n=104)	Community controls (n=93)	Univariate differences[1]
Demographic Variables				
Mean Age (SD) (years)	43.01 (14.03)	45.01 (14.23)	46.36 (17.81)	F=1.05, P=0.35
Sex (% female)	44 (52.38)	52 (50.00)	46 (49.46)	χ^2=0.17, P=0.92
Completed secondary education (%)	35 (42.68)	51 (50.49)	69 (74.19)	χ^2=19.53, P<0.001
Currently employed (%)	22 (26.51)	33 (32.67)	55 (59.14)	χ^2=22.80, P<0.001
Marital status				χ^2=24.20, P<0.001
Never married (%)	27 (32.53)	43 (41.75)	28 (30.11)	
Married/common law (%)	21 (25.30)	31 (30.10)	50 (53.76)	
Widowed/separated/divorced (%)	35 (42.17)	29 (28.16)	15 (16.13)	
Prognostic Factors				
Currently smoking (%)	35 (43.21)	35 (35.35)	11 (11.83)	χ^2=22.84, P<0.001
Taking antidepressants (%)	40 (47.61)	32 (30.77)	14 (15.05)	χ^2=22.04, P<0.001
Mean serum BDNF (SD) (ng/ml)	24.21 (7.19)	23.88 (6.95)	24.77 (7.19)	F=0.297, P=0.59
Mean body mass index (SD) (kg/m^2)	27.88 (7.28)	29.78 (9.80)	27.73 (6.18)	F=0.045, P=0.83

Psychiatric Diagnoses[2]				
Major depressive disorder (%)	42 (63.63)	38 (41.30)	20 (21.74)	χ^2=28.21, P<0.001
Mood disorder (%)	63 (95.45)	76 (82.60)	26 (28.26)	χ^2=95.22, P<0.001
Anxiety disorder (%)	43 (65.15)	49 (53.26)	9 (9.78)	χ^2=58.93, P<0.001
Alcohol abuse (%)	18 (27.27)	13 (14.13)	4 (4.35)	P<0.001
Substance abuse (%)	11 (16.67)	11 (11.96)	2 (2.17)	P=0.003
Eating disorder (%)	5 (75.76)	5 (5.43)	1 (1.09)	P=0.11
Psychotic disorder (%)	3 (4.55)	6 (6.52)	0 (0)	P=0.024
Antisocial personality disorder (%)	15 (22.73)	0 (0)	2 (2.17)	P<0.001

[1] Analysis of variance tests (ANOVA) were used to compare means for continuous variables. Chi-square tests were used to compare proportions for categorical variables. Fisher's exact test was used for categorical variables when one or more values in the contingency table were below 5.
[2] Since not all participants underwent the Mini-International Neuropsychiatric Interview (MINI), the group sizes for this section of the table are as follows: 66 cases, 92 psychiatric controls, 92 community controls.
Abbreviations: SD, standard deviation; BDNF, brain-derived neurotrophic factor.

Table 4.4.1: Association between BDNF and Key Baseline Characteristics

	Univariate Analysis			Multivariable Analysis [1]		
Variables	β Estimate	Standard Error	P-value	β Estimate	Standard Error	P-value
Group						
Suicide attempt	-0.56	1.09	0.611	0.28	1.20	0.818
Psychiatric control	-0.89	1.04	0.391	-0.82	1.10	0.455
Community control	0 (ref.)	--	--	0 (ref.)	--	--
Age (years)	-0.02	0.03	0.388	-0.02	0.03	0.578
Sex (female)	1.29	0.87	0.138	1.51	0.89	0.090
Currently smoking (yes)	1.08	0.97	0.268	1.43	1.02	0.163
Body mass index (kg/m^2)	-0.03	0.05	0.613	-0.01	0.05	0.864
Taking antidepressant (yes)	-2.06	0.92	0.026 *	-2.50	0.99	0.012 *

[1]Adjusted R-squared: 0.022
P-value: 0.09
N=250

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CHAPTER 5

Study 3

Social Risk Factors for Suicidal Behaviour Vary by Sex

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Keywords: Suicidal behaviour, sex differences, social factors, risk factors

5.1 ABSTRACT

Suicide is a leading cause of death worldwide. Social factors contribute to suicide risk, and their effects may differ between men and women. The objective of this study is to explore the sex differences in social factors for attempted suicide using a case-control study design. Individuals who attempted suicide (n=146) were compared to psychiatric and community controls (n=197). Information on sociodemographic variables and other social factors was collected upon recruitment. Logistic regression was used to assess the associations between social factors and attempted suicide. Some risk factors were found to differ between men and women. Completion of post-secondary education and religious practice were found to be significant protective factors in women but not in men. Unemployment and recent major stressful life events were significant risk factors in men but not in women. Our findings could aid clinicians in assessing suicide risk and identifying vulnerable individuals.

5.2 INTRODUCTION

Suicide is one of the leading causes of death worldwide. It claims the lives of nearly one million people each year, and has a devastating impact on families, communities, and society (1). Attempted suicide occurs 10-20 times more often than completed suicide, and is a significant risk factor for death by suicide in the general population (1, 2).

There are a number of factors that are thought to contribute to suicide risk, including biological and social factors. Known biological risk factors include psychiatric disorders (particularly mood disorders) and chronic illness (3). Social risk factors may include sociodemographic factors, as well as living alone and adverse experiences (3-6). Potentially protective social factors include religious involvement and social connectivity (7, 8).

There is some evidence that social risk factors for suicidal behaviour may differ between the sexes. Studies have shown that sociodemographic factors are stronger predictors of suicide in men than in women, including unmarried status, low education level, low income, and unemployment (3, 9). A study of over 6 000 older adults who had died by suicide found that men were more likely than women to have experienced interpersonal problems, job or legal problems, or a recent crisis (10). In a study of individuals who had attempted suicide in Japan, financial and work problems were more common among men, while family problems and loneliness were more common among women (11). The evidence generally supports the notion that men are more vulnerable to socioeconomic difficulties while women are more vulnerable to psychosocial difficulties (12, 13).

Relatively few studies have explored the topic of sex differences in risk factors for suicidal behaviour. Of those that did, some used unadjusted statistical analyses, which may have led to biased estimates (10, 11). A thorough understanding of the sex differences with regard to social risk factors for suicidal behaviour will help clinicians to identify and treat individuals at risk.

The objective of this study is to explore the sex differences in social factors for attempted suicide using a case-control study design.

5.3 METHODS

5.3.1 Data collection and study participants

The data were collected for the Study of Determinants of Suicide Conventional and Emergent Risk (DISCOVER) (14). DISCOVER is an observational matched case-control study that aims to identify the risk factors involved in suicidal behaviour. The study participants were recruited from hospitals and community settings between March 2011 and November 2014. The Hamilton Integrated Research Ethics Board (HiREB) approved this study (REB numbers 10-661 and 11-3479).

The study included men and women aged 18 or older who could provide written informed consent, communicate in English, and follow study procedures. Cases were defined as individuals who had been admitted to hospital following a serious suicide attempt with intent to die and requiring medical or psychiatric intervention. Two control groups were included. The first control group consisted of individuals with psychiatric disorders and no history of suicide attempts. The second control group consisted of individuals recruited from community and non-psychiatric clinical areas with no history of suicide attempts. While most of the cases and control participants in DISCOVER were

matched on age and sex, additional, unmatched participants were also recruited in order to increase the size of the sample. Since we included these individuals in our analyses, we did not perform matched statistical analyses.

Trained research personnel approached eligible inpatients and provided detailed information about the study. Community controls were recruited by distributing advertisements in hospitals and community settings. Upon recruitment, participants signed informed consent forms and underwent a structured interview. Data were collected on sociodemographic variables, medical history, health-related behaviours, psychopathology, and suicidal behaviour. All of the study questionnaires were compiled using previously validated diagnostic and assessment tools. For participants in the case group, a detailed description of the suicide attempt was recorded. All assessments were administered in hospital or community by trained research staff.

5.3.2 Statistical analysis

Logistic regression models were used to assess the associations between social risk factors and attempted suicide in men and women separately, and in the entire sample. The following variables were included in the logistic regression models because of their clinical relevance to suicidal behaviour: age, education, employment status, marital status, religious practice, major stressful life events, and childhood abuse. Psychiatric and community controls were combined into one group for the primary analyses. Subgroup analyses were performed in which cases (within each sex group) were compared to psychiatric and community controls separately.

R version 3.0.2 was used for all analyses (15).

5.3.3 Power analysis

The generally accepted rule of thumb for logistic regression requires a minimum of 10 events per predictor variable (16). Our sample includes 146 events (individuals who attempted suicide) (81 women and 65 men). We included 7 predictor variables in our logistic regression analysis. Therefore, we believe our analysis has adequate power to detect important differences.

The reporting of this study follows the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines (17).

5.4 RESULTS

The study recruited a total of 343 participants, including 146 cases, 104 psychiatric controls, and 93 community controls. The mean time since the suicide attempt for all cases was 1.5 months. The recruitment process is summarized in Figure 5.4. The characteristics of the sample are summarized in Table 5.4. The mean age of the participants was 45.45 years (SD=15.43). Approximately half of the participants were female (52.19%). No significant differences between cases and controls were found in age or sex.

5.4.1 Primary analysis

The results of the logistic regression, including odds ratios (OR), 95% confidence intervals (CI) and p-values, are presented in Table 5.4.1.

In women, being on disability (OR 6.12, 95% CI 2.36-16.96, $p<0.001$), and being widowed, separated, or divorced (OR 3.11, 95% CI 1.06-9.59, $p=0.042$) were significantly associated with increased risk of attempted suicide. Post-secondary

education (OR 0.30, 95% CI 0.14-0.64, p=0.002) and religious practice were associated with decreased risk of attempted suicide (OR 0.43, 95% CI 0.19-0.92, p=0.031).

In men, factors that were significantly associated with increased risk of attempted suicide included being unemployed (OR 4.31, 95% CI 1.44-13.72, p=0.01) or on disability (OR 3.04, 95% CI 1.04-9.20, 0.04); being widowed, separated, or divorced (OR 3.49, 95% CI 1.06-12.32, p=0.044); and experiencing a major stressful event in the past year (OR 4.71, 95% CI 1.58-16.61, p=0.009).

5.4.2 Subgroup analyses

In both men and women, comparing individuals who had attempted suicide to psychiatric and community controls separately revealed that most of the differences were only between the cases and community controls. In women, of the factors found to be significantly associated with attempted suicide risk in the primary analysis, only post-secondary education (OR 0.38, 95% CI 0.15-0.90, p=0.03) was a significant protective factor when community controls were excluded from the model. When cases were compared to only community controls, all factors that reached significance in the primary analysis remained significant.

In men, none of the factors found to be significantly associated with attempted suicide in the primary analyses were significant when cases were compared to just psychiatric controls. When cases were compared to community controls, unemployment and major stressful events remained significant risk factors.

5.5 DISCUSSION

In this study we used multivariable logistic regression models to investigate the associations between a variety of social factors and suicidal behaviour in men and women.

Our primary analyses revealed that some of the social risk factors for attempted suicide differ between men and women. Completion of post-secondary education and practicing religion were found to be significant protective factors in women but not in men. Unemployment and experiencing a major stressful life event in the past year were significant risk factors in men but not in women.

Our finding that religious practice was protective only in women is consistent with evidence of a stronger protective effect of religiousness on mental and physical well-being in women than in men (18, 19). Our finding that unemployment was a significant risk factor only in men is consistent with previous literature showing that men respond more adversely than women to poor economic conditions (3, 9, 12). As well, our finding of a significant association between stressful life events and suicidal behaviour only in men is supported by evidence of a stronger relationship between stressful life events and suicide in men (20). Furthermore, it is possible that in women, the effects of stressful life events are more often mitigated by seeking professional help (21) or drawing on support systems (22).

Our subgroup analyses showed that the major differences found in the primary analyses were in fact between individuals who had attempted suicide and community controls. When community controls were excluded from the models, few significant risk factors remained. This underscores the importance of including psychiatric control

groups in studies of suicidal behaviour. Many of the known risk factors for suicidal behaviour have been identified in studies that compared individuals who had engaged in suicidal behaviour to members of the general population (3, 4, 7, 11). Since having a psychiatric disorder is a very important risk factor for suicidal behaviour, studies that do not include a psychiatric control group may produce misleading findings.

Our study has a number of strengths, including its large sample size and inclusion of both psychiatric and community controls. We recruited individuals who had made suicide attempts with specific intent to die, and did not include individuals who had engaged in non-suicidal self-harm. We performed adjusted analyses and explored a wide variety of social risk factors for suicidal behaviour.

Our study is limited by its cross-sectional design, which precludes us from drawing inferences about causal relationships. Another limitation is that due to the inclusion of past cases of attempted suicide in addition to recent cases, some of the variables measured may have changed since the time of the attempt. For example, our study questionnaire asked participants to list stressful life events that had occurred within the past year. For individuals whose suicide attempts occurred earlier than a year prior to recruitment, their responses would not have reflected the period of time surrounding their attempts. Similarly, questions about employment status or marital status may have elicited responses that differed from what they would have been closer to the time of the past attempt.

While sex may be considered in clinical assessments of suicide risk (as men are more likely to die by suicide while women are more likely to attempt suicide (2)), clinicians should keep in mind the differing effects of other risk factors between the

sexes. An appreciation of these differences could help clinicians identify individuals who are most vulnerable toward suicidal behaviour. Additional well-powered studies of social risk factors for suicidal behaviour and the sex differences within them will improve both our understanding of this complex phenomenon and our efforts to prevent it.

5.6 ACKNOWLEDGEMENTS, COMPETING INTERESTS, AND AUTHOR CONTRIBUTIONS

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The authors declare that they have no competing interests.

RE developed the research question, analyzed and interpreted the data, and wrote and critically revised the manuscript. SP contributed to data analysis and interpretation, and critically revised the manuscript. MB and BD assisted with recruitment and critically revised the manuscript. WE was the research assistant for this study and was responsible for recruitment, interviews, and data collection. JD was the study coordinator responsible for the daily running of the study, training research assistants and study personnel on study-related procedures. SR was the study project manager responsible for the study conduct; with ZS she designed the study protocol, provided supervision of study personnel, and coordinated the overall study management. HS and EI are nurses specializing in psychiatry; they recruited hospitalized study patients, conducted interviews, collected blood samples, and provided feedback on participant recruitment and study procedures. PM was responsible for database management, quality and edits, checks of data, and provision of reports, including age and sex matching status, to the study team for the weekly study meetings. SI was responsible for data management and preparation of data reports to the team. MD designed the dietary tool and analyzed the dietary data for the DISCOVER study. JB provided advice on study procedures including

recruitment and the inclusion of psychiatric controls based on her expertise in emergency psychiatry and suicide prevention. RA and LM critically revised the manuscript. LT was responsible for the case-control study design, and overall methodological and statistical aspects of the study. ZS conceived the study and was principally responsible for the conduct of the study and obtaining funding. ZS also developed the research question, contributed to data analysis and interpretation, and critically revised the manuscript. All authors have reviewed and approved the final manuscript.

5.7 FIGURES AND TABLES

Figure 5.4: Flow diagram for recruitment of participants

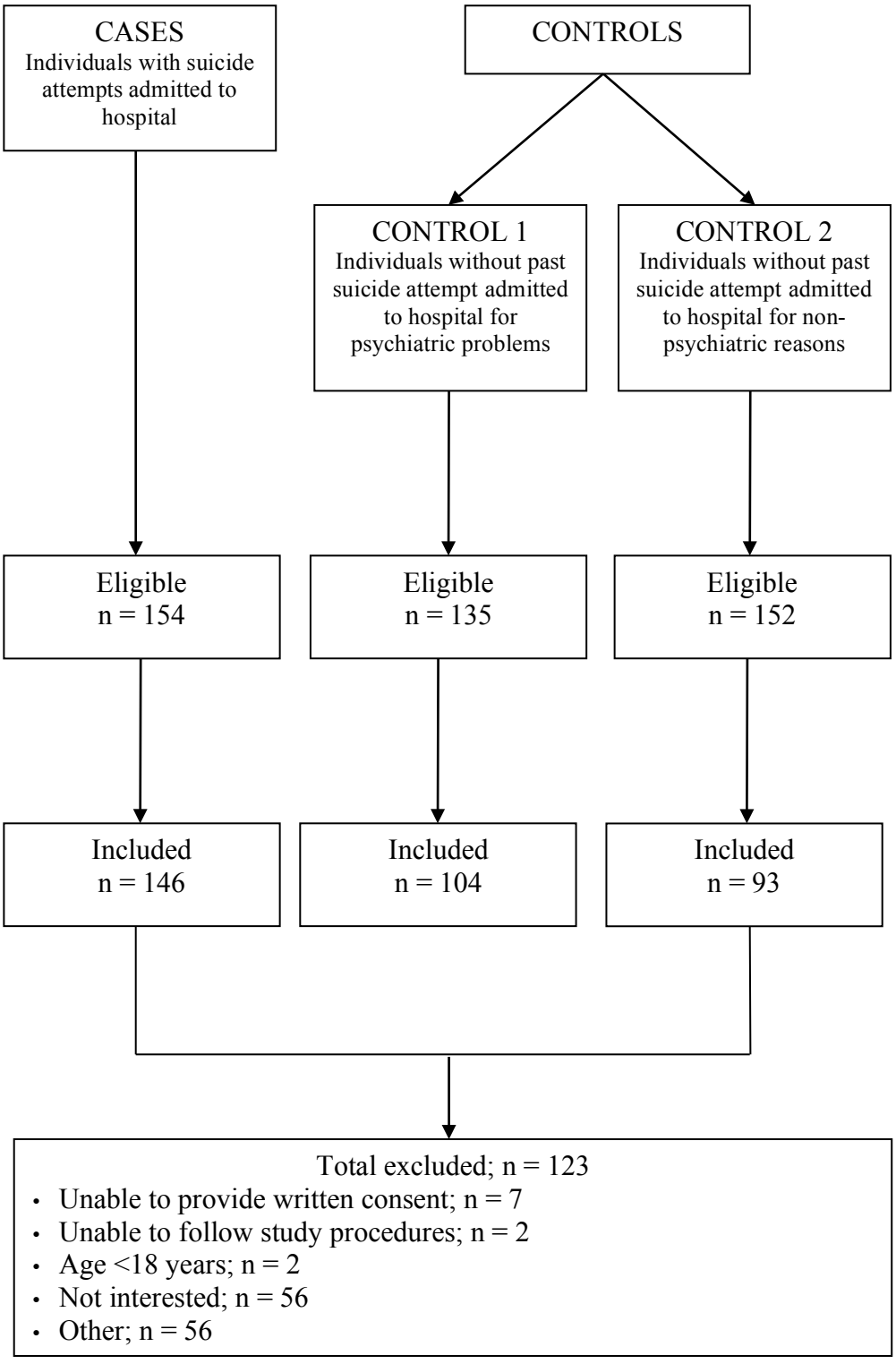

Table 5.4: Baseline Sample Characteristics

	Cases (n=146)	Psychiatric controls (n=104)	Community controls (n=93)	All controls (n=197)
Age (years): Mean (SD)	45.18 (14.70)	45.01 (14.23)	46.36 (17.81)	45.65 (15.99)
Sex (% female)	81 (55.48)	52 (50.00)	46 (49.46)	98 (49.75)
Ethnicity (% Caucasian)	135 (92.47)	81 (79.41)	61 (65.59)	143 (72.82)
Completed post-secondary education (%)	58 (40.28)	51 (50.49)	69 (74.19)	120 (61.86)
Employment status				
Employed (%)	37 (25.34)	36 (34.62)	57 (61.29)	93 (47.21)
Unemployed (%)	29 (19.86)	21 (20.19)	10 (10.75)	31 (15.74)
Retired (%)	17 (11.64)	7 (6.73)	20 (21.5)	27 (13.71)
On disability (%)	56 (38.36)	28 (26.92)	3 (3.23)	31 (15.74)
On social security (%)	7 (4.79)	9 (8.65)	0 (0)	9 (4.57)
Marital status				
Never married (%)	45 (31.03)	43 (41.75)	28 (30.11)	71 (36.22)
Married/common law (%)	39 (26.90	31 (30.10)	50 (53.76)	81 (41.33)
Widowed/separated/divorced (%)	61 (42.07)	29 (28.16)	15 (16.13)	44 (22.45)
Living alone (%)	64 (46.04)	47 (46.08)	27 (29.03)	37.95
Practice religion (%)	67 (50.38)	57 (58.16)	62 (66.67)	119 (62.39)
Social Support (% satisfied)	115 (92.74)	80 (85.11)	90 (98.90)	170 (91.89)
Experienced major life stress(es) in past year	117 (89.31)	84 (89.36)	46 (49.46)	130 (69.52)
Experienced childhood abuse (%)	79 (59.85)	47 (50.54)	21 (22.58)	68 (36.56)
Bullied as a child (%)	66 (50.77)	45 (48.39)	29 (31.18)	74 (39.78)

Table 5.4.1: Association between Social Factors and Attempted Suicide

	Women (n=162)			Men (n=147)			Entire sample (n=309)		
Variables	**OR**	**95% CI**	**P**	**OR**	**95% CI**	**P**	**OR**	**95% CI**	**P**
Age (years)	0.99	0.95-1.01	0.192	1.01	0.97-1.05	0.760	0.99	0.96-1.01	0.327
Completed post-secondary education	0.30	0.14-0.64	0.002**	0.66	0.29-1.50	0.323	0.48	0.28-0.82	0.007**
Employment status									
Employed (%) (ref.)	1.00	--	--	1.00	--	--	1.00	--	--
Unemployed (%)	0.84	0.26-2.61	0.771	4.31	1.44-13.72	0.011*	2.00	0.95-4.25	0.070
Retired (%)	1.23	0.29-5.04	0.778	2.43	0.61-10.11	0.211	1.78	0.68-4.62	0.236
On disability (%)	6.12	2.36-16.96	<0.001***	3.04	1.04-9.20	0.044*	4.33	2.19-8.76	<0.001***
On social security (%)	0.30	0.01-2.44	0.321	1.62	0.22-11.81	0.621	0.66	0.16-2.41	0.544
Marital status									
Never married (ref.)	1.00	--	--	1.00	--	--	1.00	--	--
Married	2.89	0.97-9.05	0.061	0.81	0.25-2.61	0.726	1.56	0.73-3.38	0.256
Widowed/separated/ divorced	3.11	1.06-9.59	0.042*	3.49	1.06-12.32	0.044*	3.11	1.45-6.86	0.004**
Practice religion	0.43	0.19-0.92	0.031*	0.75	0.32-1.76	0.507	0.60	0.35-1.03	0.066
Major stresses	2.20	0.80-6.40	0.134	4.71	1.58-16.61	0.009**	3.08	1.53-6.50	0.002**
Childhood abuse	1.15	0.51-2.56	0.725	1.79	0.79-4.07	0.162	1.44	0.84-2.49	0.184

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CHAPTER 6

6.1 THESIS CONCLUSION

6.1.1 Overview

While the causes of suicidal behaviour may never be fully understood, studies of its potential determinants can provide valuable knowledge that will lead to improved clinical assessment and prevention methods. This thesis contributes meaningfully to the literature in the area of suicidal behaviour. We have explored the role of BDNF levels as a possible biomarker for suicidal behaviour, both by systematically reviewing the literature and by analyzing clinical data. We have also explored the social risk factors for suicidal behaviour and the sex differences within them. This work concludes by identifying areas requiring further research.

6.1.2 BDNF and suicidal behaviour

In Study 1 we conducted the first systematic review of the literature on BDNF and suicidal behaviour. Our review and meta-analysis revealed that the evidence does not provide consistent support for an association between BDNF and suicidal behaviour. The 14 studies included in the qualitative review varied in their findings. The meta-analysis of three studies indicated that there is not a significant association between serum BDNF and attempted suicide. Overall, the studies in this area are subject to a number of methodological limitations. Our review indicates that additional, methodologically rigorous studies will help to determine the relationship between BDNF levels and suicidal behaviour.

In Study 2, we conducted the largest study to date exploring the association between serum BDNF level and attempted suicide. In our large sample of individuals

who had made suicide attempts, psychiatric controls, and community controls, linear regression analyses revealed that serum BDNF level was not significantly associated with attempted suicide.

While there is some evidence to support the role of BDNF as a potential biomarker for suicidal behaviour, based on the findings of Studies 1 and 2, currently the evidence does not warrant using measures of BDNF in a clinical setting to assess suicide risk. However, our findings from Study 2 should be replicated in a large cohort study.

6.1.3 Social factors and suicidal behaviour

In Study 3, we explored the relationship between social factors and attempted suicide. Our logistic regression analyses revealed sex differences with regard to several risk factors. Some factors were shown to be significantly associated with attempted suicide only in women (education level and religious practice), while others were significant only men (unemployment and stressful life events).

Sex differences in risk factors for suicidal behaviour is a relatively understudied area. Further research should build upon our findings and aim to shed light on additional sex differences within both social and biological risk factors for suicidal behaviour.

6.1.4 Future directions

Future studies of determinants of suicidal behaviour should aim to overcome the methodological limitations found in many of the existing studies. To date, the majority of studies of BDNF and suicidal behaviour have used small clinical samples, which likely led to inadequate statistical power. As well, most studies used univariate statistical tests to compare BDNF levels between groups of individuals with and without suicidal

behaviour. Future studies of this relationship should use large samples and should adjust for confounding variables in their analyses in order to produce unbiased estimates.

Research should also explore a range of potential suicide biomarkers. While the evidence (however limited) currently does not point to an independent association between BDNF and suicidal behaviour, there are numerous other biological substances that may prove fruitful in understanding and predicting suicidal behaviour. These include neurotransmitters and genetic variants.

Within the literature on social risk factors for suicidal behaviour, a prevalent limitation is a lack of psychiatric comparator group. Many studies have compared individuals who have attempted or completed suicide with a control group derived from the general population. Since most incidents of suicidal behaviour occur in the context of a psychiatric disorder, the findings of those studies may be misleading. Future studies that include both psychiatric and community comparator groups will help to produce a clear picture of the social determinants of suicidal behaviour.

Studies should also endeavor to explore the interactions among biological and social factors for suicidal behaviour. Since any act of suicidal behaviour might be attributed to an intricate combination of internal and external factors, research efforts aimed at understanding this interplay may reveal interesting and meaningful findings.

The ultimate goal of research on this topic is to increase our ability to identify individuals at increased risk of suicidal behaviour and provide effective preventative interventions. It is our hope that studies like those described in this work will move us closer to that goal.

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SYSTEMATIC REVIEWS

PROTOCOL Open Access

Association between BDNF levels and suicidal behaviour: a systematic review protocol

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Abstract

Background: Suicide is a worldwide public health concern that claims close to 1 million lives each year. Suicidal behaviour is a significant risk factor for completed suicide and is much more prevalent than completed suicide. Many internal and external factors contribute to the risk of suicidal behaviour. Recent research has focused on biological markers in suicide risk, including brain-derived neurotrophic factor (BDNF). BDNF is a protein involved in the growth, function, and maintenance of the nervous system. It has been implicated in psychiatric disorders and suicide. While some evidence suggests that reduced levels of BDNF are associated with suicide, the precise relationship has yet to be determined. The aim of this study is to review the literature examining the relationship between levels of BDNF and suicidal behaviour.

Methods: A predefined search strategy will be implemented to search the following electronic databases: PubMed/MEDLINE, Excerpta Medica Database (EMBASE), PsycINFO, and Cumulative Index to Nursing and Allied Health Literature (CINAHL) from inception. The articles will be screened by two independent authors (RE and SP) using predetermined inclusion and exclusion criteria. Discrepancies will be resolved by consensus, or by a third author (ZS) in cases of disagreement. The primary outcome will be the association between levels of BDNF and suicidal behaviour. A meta-analysis will be conducted if appropriate. Quality of evidence and risk of bias will be evaluated.

Discussion: The findings of this review will assist in identifying and treating individuals at increased risk of suicide.

Systematic review registration: PROSPERO CRD42015015871.

Keywords: Suicide, Attempted suicide, Suicidal ideation, Brain-derived neurotrophic factor, Systematic review, Protocol

Background

Suicide is a worldwide public health concern. It claims the lives of over 800,000 people each year, and the numbers continue to increase [1]. Suicide has a devastating impact at a number of levels, including the individual, family, community, and society. Suicidal behaviour encompasses a complex set of ideas, plans, and acts intended to end one's own life. It occurs 10 to 20 times more often than completed suicide and is a significant risk factor for completed suicide in the general population [1,2].

While the causes of suicide are unclear, a number of internal (biological and psychological) and external (social and environmental) factors are thought to contribute to the risk of suicidal behaviour. Internal risk factors include psychiatric disorders (especially mood disorders), substance use disorders, chronic illness, and demographic variables (age and sex) [3]. External factors include unemployment, unmarried status, and a lack of social support [2,3]. While having a psychiatric disorder significantly increases one's risk of suicide [4], and about 90% of people who attempt or complete suicide have a psychiatric disorder [3], most individuals with psychiatric

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disorders never attempt suicide. This indicates that there may be a predisposition toward suicidal behaviour independent of the underlying psychiatric disorders [2]. As well, many cases of suicide cannot be explained by the conventional risk factors proposed by clinical and research observations. Therefore, the focus has shifted to the investigation of biological markers in suicide risk, which has become more common among the recent literature.

The role of neurotrophins has been explored in relation to psychiatric disorders, including depression, bipolar disorder, anxiety, and schizophrenia [5]. Neurotrophins are a family of proteins that regulate the survival, development, maintenance, and function of vertebral nervous systems [6]. Brain-derived neurotrophic factor (BDNF) is the most abundant member of the neurotrophin family [6] and has been implicated in both suicide and suicidal behaviour [7]. BDNF is expressed in the brain and in other body tissues such as skeletal muscle and circulates throughout the body in the bloodstream [7,8]. When BDNF is released by a cell, it triggers a cascade of events that lead to neurogenesis, nerve growth, neuroplasticity, and neurotransmission [7]. BDNF is also important in morphological plasticity, neurite outgrowth, phenotypic maturation, and protein synthesis for neuron and synaptic functioning [6]. Since BDNF is intrinsic to these important processes, pathological changes in BDNF levels are likely involved in neurological deficits that impair one's ability to adapt to difficult situations. Altered levels of BDNF may be responsible for the cognitive deficits and altered brain structure associated with depression, stress, and suicide.

Some studies have linked reduced levels of BDNF to psychiatric disorders and suicide. Low levels of serum BDNF are associated with a dispositional vulnerability to depression and with acute depressive states in the general population [9]. Lower levels of both serum and plasma BDNF are associated with major depressive disorder, and serum levels in particular have been correlated to severity of depression [10,11]. Stress, which plays an important role in suicidal behaviour and constitutes a major risk factor [7,12], is associated with altered levels of BDNF in the brain [13-16]. But while brain levels of BDNF are altered in depression and stress, evidence suggests a differential role of BDNF depending on the location in the brain. Depression and stress are associated with low levels of BDNF in the hippocampus and prefrontal cortex but high levels of BDNF in the amygdala and nucleus accumbens [17]. Antidepressants have been shown to normalize levels of BDNF expression [18].

Postmortem studies of the brains of suicide victims have revealed abnormally low levels of BDNF and its receptor, tropomyocin receptor kinase B (TrkB), compared to controls [19,20]. Interestingly, this was true regardless of the psychiatric diagnosis. Other studies have measured peripheral levels of BDNF in the blood of suicidal individuals. Deveci and colleagues compared serum BDNF levels among suicide attempters, depressed patients, and healthy controls [21]. Mean serum BDNF was significantly lower in both the suicidal group and the depressed group compared to the controls. Studies of plasma BDNF have found decreased levels in suicidal depressed patients compared with nonsuicidal depressed patients [22,23].

BDNF is a modifiable risk factor for suicidal behaviour. However, relatively few studies have investigated the relationship between levels of BDNF and suicidal behaviour. As well, since BDNF levels are altered in both depression and suicide, it is unclear whether the differences are related specifically to suicide. A systematic review is needed in order to summarize the existing studies and determine whether BDNF levels are in fact associated with suicidal behaviour, as well as to identify gaps in the literature that require further research.

Objectives

The objective of this systematic review is to elucidate the association between levels of BDNF and suicidal behaviour (including completed suicide, attempted suicide, and suicidal ideation) in an adult population through a methodological summary of the literature.

The study goals are the following:

1. To investigate the relationship between levels of BDNF and suicidal behaviour by summarizing primary studies that have examined this relationship.
2. To combine the results of primary studies in a statistical manner using meta-analysis, when appropriate.
3. To critically evaluate the existing literature and identify which areas require additional research.

Methods/Design

Inclusion and exclusion criteria

This systematic review will include published observational studies (case–control and cohort studies) of central and peripheral levels of BDNF (including postmortem brain tissue, cerebrospinal fluid, plasma, serum, whole blood, and urine) and suicidal behaviours (including completed suicide, attempted suicide, and suicidal ideation) in a population aged 18 and older. Included studies will have investigated the association between levels of BDNF and suicidal behaviour by comparing BDNF levels between groups with and without suicidal behaviour. This review will include clinical samples as well as community-based samples. No demographic limitations will be applied apart from age, and no special populations will be excluded (e.g. incarcerated individuals, pregnant women, etc.).

Search strategy

All relevant studies will be identified with no language or time restrictions. The databases to be searched from

inception are as follows: PubMed/MEDLINE, PsycINFO, Excerpta Medica Database (EMBASE), and Cumulative Index to Nursing and Allied Health Literature (CINAHL). The search strategy (presented in Table 1) will use relevant keywords and their associated medical subject headings (MeSH). A number of different search terms related to suicidal behaviour that are common in the literature will be used in order to encompass this broad topic, including

Table 1 Search strategy for retrieval of relevant articles from multiple databases

Database	Search strategy
MEDLINE ($n = 106$)	1. exp Suicide/
	2. suicid*.mp.
	3. exp Self-Injurious Behavior/
	4. (self harm* or self inflict* or self injur* or self wound* or self mutilat*).mp.
	5. automutilat*.mp
	6. 1 or 2 or 3 or 4 or 5
	7. brain derived neurotrophic factor.mp. or Brain-Derived Neurotrophic Factor/
	8. bdnf.mp
	9. 7 or 8
	10. 6 and 9
EMBASE ($n = 366$)	1. exp suicidal behaviour/
	2. suicid*.mp.
	3. exp automutilation/
	4. (self harm* or self inflict* or self wound* or self mutilat* or autmutilat*).mp.
	5. 1 or 2 or 3 or 4
	6. brain derived neurotrophic factor.mp. or exp brain derived neurotrophic factor/
	7. bdnf.mp.
	8. 6 or 7
	9. 5 and 8
PsycINFO ($n = 76$)	1. exp Suicide/
	2. exp Attempted Suicide/
	3. exp Suicidal Ideation/
	4. suicide*.mp
	5. exp Self Injurious Behavior/
	6. (self harm* self injur* or self inflict* or self wound* or self mutilat* or autmutilat*).mp.
	7. 1 or 2 or 3 or 4 or 5 or 6
	8. brain derived neurotrophic factor.mp. or exp Brain Derived Neurotrophic Factor/
	9. bdnf.mp.
	10. 8 or 9
	11. 7 and 10
CINAHL ($n = 4$)	1. MH ("Suicide+")
	2. "suicid*"
	3. MH ("Self-Injurious Behavior")
	4. "self harm*" OR "self injur*" OR "self inflict*" OR "self wound*" OR "self mutilat*" OR "automutil*"
	5. 1 or 2 or 3 or 4
	6. "brain derived neurotrophic factor"
	7. "bdnf"
	8. 6 or 7
	9. 5 and 8

CINAHL, Cumulative Index to Nursing and Allied Health Literature; EMBASE, Excerpta Medica Database.

"suicide", "attempted suicide", "self-injurious behaviour", "self harm*", "automutilation", "self inflict*", and "suicidal ideation." These terms will be combined with the term "brain-derived neurotrophic factor" or "BDNF". The search will include titles, abstracts, and keyword fields. The reference lists from the included articles will be scanned manually to identify additional studies. The grey literature will be searched using the ProQuest Dissertations and Theses Database. Reviews, abstracts, and commentaries will be excluded. No language restrictions will be applied. An experienced health sciences librarian (LB) was consulted and assisted in the search strategy. A search alert will be set up to ensure the retrieval of relevant studies published after the initial search.

Data screening

All citations and abstracts retrieved using the predefined search strategy (Table 1) will be screened by two raters (RE and SP) independently. Eligible articles will be identified using pre-established criteria and retrieved for full-text review. Disagreements at any point in the review process will be resolved by discussion. In cases where consensus is not reached, eligibility will be determined by a third author (ZS). Studies that are ineligible will be excluded from review. The reasons for exclusion will be recorded and described in the flow diagram (see Additional file 1: Figure S1). For each phase of screening, the Kappa statistic will be used to calculate inter-rater agreement [24]. The authors of the studies will be contacted for clarification and additional data when necessary.

Data extraction

The two raters (RE and SP) will extract data independently from the included studies using a pre-established data extraction form that will be pilot tested beforehand (see Additional file 2). The raters will obtain the following information from each study: first author, year of publication, city and country of publication, article title, journal, study design, description of sample population, mean age, ethnicity, and definition of suicidal behaviour. For studies that include more than one measure of suicidal behaviour, each measure will be recorded. This will allow for the combination of studies with the same measures in a meta-analysis. For example, some studies have used the suicide item in Hamilton Depression Rating Scale (HDRS), while others have used the Beck Scale for Suicidal Ideation (BSS) [25,26]. We will record all measures reported so that we can combine, for instance, all studies that used the BSS. We expect most studies to report suicidal behaviour as a dichotomous measure (e.g. history of suicide attempt or no history of suicide attempt). However, if studies used different measurement scales to indicate severity of suicidal behaviour, then we will use a dichotomized outcome based on the presence of suicidal behaviour, regardless of severity. For studies that report multiple time points for suicidal behaviour (e.g. suicide attempt within the last month vs. the past 3 months vs. lifetime), all time points will be recorded. This will allow for the combination of similar time frames when possible. Information regarding the BDNF measurements will be obtained, including the tissue sample in which it was measured, the lab analysis methods, the mean BDNF levels and standard deviations, and the unit of measurement used. If relevant, the comparison group and any underlying psychiatric disorders will also be recorded. For each study, primary and secondary outcome measures, results, statistical analyses, and conclusions will be recorded. If any data are missing or incomplete, authors will be contacted for additional details.

Assessment of quality

The risk of bias of included studies will be assessed by two independent raters (RE and SP) using the Newcastle-Ottawa Scale (NOS) [27]. An adapted version of the NOS will be used, in keeping with previous systematic reviews of observational studies (see Additional file 3) [28]. This version of the NOS contains seven questions in the following domains: methods for selecting study participants (selection bias), methods to control for confounding (performance bias), statistical methods (detection bias), and methods of exposure and outcome assessment (information bias). Risk of bias will be assessed on a scale from 0 to 3, where 0 indicates high risk of bias and 3 indicates low risk. Descriptions and examples of high and low risk of bias are provided. This adapted NOS also includes categories related to statistical methods, confounding effects, and reporting of missing data. The Grading of Recommendations, Assessment, and Evaluation (GRADE) framework will be used to report the quality of evidence and strength of recommendations [29]. This framework provides a systematic approach for considering and reporting risk of bias, imprecision, inconsistency, indirectness of study results, and publication bias. A summary of findings table will be presented to allow for assessment of confidence in the estimates.

Statistical analyses and heterogeneity

The results of this systematic review will be presented as a qualitative summary of the literature. When possible, meta-analyses will be performed. This review will encompass a wide variety of studies with different designs, sample populations, BDNF measurements, and definitions of suicidal behaviour. Clinical and methodological heterogeneity are expected. Therefore, separate meta-analyses will be conducted on groups of studies that share the following characteristics:

1. Study design (e.g. case control vs. cohort)
2. Definition of suicidal behaviour (completed suicide, attempted suicide, or suicidal ideation)

3. Type of tissue from which BDNF was sampled (e.g. plasma, serum, brain tissue)

Meta-analyses will be performed using the extracted data from groups of studies if the following conditions are met:

- More than one study is found that share all of the characteristics listed above
- There are minimal differences among the studies in other relevant characteristics (such as sample population)
- Data in each study are available and reported with sufficient detail.

Heterogeneity will be assessed using the I^2 statistic. The interpretation of the I^2 value will be based on the guidelines in the Cochrane Handbook for Systematic Reviews of Interventions, which defines 0% to 40% as low heterogeneity, 30% to 60% as moderate heterogeneity, 50% to 90% as substantial heterogeneity, and 75% to 100% as considerable heterogeneity [30]. In this study, an I^2 value below 50% will be considered low heterogeneity. The P value from the chi-squared test will also be taken into consideration, with significant heterogeneity being defined with a P value below 0.10. Groups of studies in which heterogeneity is found to be low ($I^2 < 50\%$) will be assessed in a combined statistical manner using meta-analysis. The mean differences (MD) in the BDNF level between groups with and without suicidal behaviour will be combined into a summary estimate. Only adjusted values extracted from the primary studies will be used. A random-effects model will be implemented, as it accounts for both within-study and between-study variability. As well, a mixed-effects model will be used to examine the possible mediation effect of BDNF on the relationship between other variables (including sex, age, and psychiatric diagnosis) and risk of suicidal behaviour. Sensitivity analysis will be conducted based on risk of bias; studies with a score of 0 on the NOS will be excluded to determine whether the summary estimate stays the same.

The main source of heterogeneity hypothesized is clinical heterogeneity, resulting from diversity in the populations being studied. Some studies have derived their samples from particular clinical populations, such as depressed patients, while others have sampled populations with a range of psychiatric diagnoses, or community-based populations. Since alterations in BDNF levels are linked to psychiatric disorders, particularly depression, the sample characteristics could have a significant influence on the associations between BDNF levels and suicidal behaviour [5,9]. The implications of this heterogeneity on the interpretation of the results will be discussed.

In the event that the heterogeneity is too high to allow for meta-analyses to be performed, the results of this systematic review will be presented as a narrative summary of the literature examining the relationship between levels of BDNF and suicidal behaviour. The included studies will be synthesized in a comprehensive, up-to-date review of this emerging area of research.

Presenting and reporting of results

This systematic review will be performed and presented according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, as well as the Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines [31,32]. The article selection process will be summarized in a flow diagram (see Additional file 1: Figure S1). The relevant outcomes and characteristics of each study will be reported in summary tables. Publication bias will be assessed using Egger's plot.

This protocol follows the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) 2015 Statement [33].

Discussion

This systematic review will present evidence from which conclusions can be made regarding the relationship between levels of BDNF and suicidal behaviour. It is expected that an inverse correlation will be found, with reduced levels of BDNF associated with suicidal behaviour. The findings of this systematic review will contribute to our understanding of BDNF as a biological factor involved in suicide risk, and of suicide pathology more generally. These findings, as well as the appraisal of the status of the literature, will be of use to clinicians, in identifying individuals at increased risk of suicide, and researchers, in developing therapeutic targets.

Additional files

Additional file 1: Figure S1. PRISMA flow diagram.
Additional file 2: Data extraction form.
Additional file 3: Modified NOS.

Abbreviations

BDNF: brain-derived neurotrophic factor; TrkB: tropomyocin receptor kinase B; NOS: Newcastle-Ottawa Scale.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

RE conceived and designed the study, wrote and revised critically the manuscript, devised the data extraction form, and approved the final manuscript. SP assisted in drafting the manuscript; participated in the methodology, interpretation, and critical revision of the manuscript; and approved the final manuscript. MB participated in the critical revision, developed the quality assessment tool, and approved the final manuscript. LB participated in the development of the search strategy and approved the final manuscript. RA participated in the critical revision and methodology and approved the final manuscript. LM participated in the critical revision

and methodology and approved the final manuscript. ZS conceived and designed the study, participated in the methodology and critical revision, and approved the final manuscript. All authors read and approved the final manuscript.

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Systematic Reviews

RESEARCH Open Access

Association between BDNF levels and suicidal behaviour: a systematic review and meta-analysis

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Abstract

Background: Suicidal behaviour is a complex phenomenon with a multitude of risk factors. Brain-derived neurotrophic factor (BDNF), a protein crucial to nervous system function, may be involved in suicide risk. The objective of this systematic review is to evaluate and summarize the literature examining the relationship between BDNF levels and suicidal behaviour.

Methods: A predefined search strategy was used to search MEDLINE, EMBASE, PsychINFO, and CINAHL from inception to December 2015. Studies were included if they investigated the association between BDNF levels and suicidal behaviours (including completed suicide, attempted suicide, or suicidal ideation) by comparing BDNF levels in groups with and without suicidal behaviour. Only the following observational studies were included: case-control and cohort studies. Both clinical- and community-based samples were included. Screening, data extraction, and risk of bias assessment were conducted in duplicate.

Results: Six-hundred thirty-one articles were screened, and 14 were included in the review. Three studies that assessed serum BDNF levels in individuals with suicide attempts and controls were combined in a meta-analysis that showed no significant association between serum BDNF and suicide attempts. The remaining 11 studies were not eligible for the meta-analysis and provided inconsistent findings regarding associations between BDNF and suicidal behaviour.

Conclusions: The findings of the meta-analysis indicate that there is no significant association between serum BDNF and attempted suicide. The qualitative review of the literature did not provide consistent support for an association between BDNF levels and suicidal behaviour. The evidence has significant methodological limitations.

Systematic review registration: PROSPERO CRD42015015871

Keywords: Suicide, Attempted suicide, Suicidal ideation, Brain-derived neurotrophic factor, Systematic review, Meta-analysis

Background

Suicide is a growing public health concern. Worldwide, over 800,000 people die by suicide every year, and the numbers are increasing [1]. Suicide affects not only the individual but the family, community, and society in which it occurs. Non-fatal suicidal behaviours, which refer to a complex set of thoughts, plans, and acts intended to end one's life, are significant risk factors for completed suicide and occur 10–20 times more often than completed suicide [1, 2].

A multitude of factors are thought to contribute to the risk of suicidal behaviour, including internal (biological and psychological) and external (social and environmental) factors. Examples of internal risk factors include psychiatric disorders, substance-use disorders, chronic illness, and demographic variables (such as older age and female sex) [3]. External risk factors can include unmarried status, unemployment, and a lack of social support [2, 3]. Most suicidal behaviour occurs in the context of a psychiatric disorder (90 % of attempted or completed suicides), but most individuals with psychiatric disorders never

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attempt suicide [3, 4]. In addition, many cases of suicide cannot be explained by the conventional risk factors that have been proposed by research and clinical observations. Consequently, there is a need to identify predictors of suicidal behaviour beyond the known risk factors [2].

Recent research has focused on biological markers of suicide risk, such as genetic variants and circulating proteins [5]. One such protein is brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family of proteins. BDNF is found in the brain and throughout the body in the bloodstream [6]. It is crucial to a number of neural processes, such as neurogenesis, neuroplasticity, and neurotransmission [6, 7].

Altered BDNF levels may play a role in the pathogenesis of suicidal behaviour by resulting in long-term changes in the brain that can lead to neuropsychological deficits. A number of studies have shown that changes in brain structure and function may be associated with depression, stress, and suicidal behaviour. These changes include reductions in neuron cell number, density, and size, as well as decreased cortical thickness and changes in synaptic circuitry [8–11]. Other studies demonstrate cognitive deficits in stress and depression [12]. This evidence supports a new hypothesis that links the pathogenesis of suicidal behaviour and depression to altered neural plasticity, which impairs the brain's ability to respond appropriately to environmental stimuli [13, 14]. It is hypothesized that pathological changes in BDNF levels are distally responsible for these neuropsychological deficits associated with depression, stress, and suicide [6].

It is also possible that short-term changes in BDNF levels may be involved in suicidal behaviour pathogenesis. There is evidence that BDNF levels can undergo short-term variations in response to external stimuli. Serum BDNF levels have been shown to increase following a 3-month reduced-calorie diet [15] and endurance training [16]. Antidepressant treatment in depressed individuals normalizes low levels of BDNF [17]. Alcohol and tobacco use have also been linked to altered levels of BDNF; excessive drinkers tend to have lower serum BDNF levels, and current smokers tend to have higher serum BDNF levels [18]. These and other variables may explain variations in BDNF level between and within individuals over time. These factors may also be related to risk of suicidal behaviour and could explain how BDNF might be related to suicidal behaviour in a proximal manner. However, there is no conclusive evidence linking short-term changes in BDNF to suicidal behaviour.

While BDNF is primarily produced in the central nervous system, it also expressed in peripheral tissue in smooth muscle cells, endothelial cells, endocrine cells, and immune cells [18]. It has been shown to cross the blood-brain barrier, and blood levels of BDNF are reflective of brain levels [19]. Brain levels of BDNF can be measured in postmortem brain tissue, and circulating levels can be measured in the blood (serum or plasma) and cerebrospinal fluid (CSF) of living individuals.

Altered central and peripheral BDNF levels have been implicated in both depression [20–22] and stress [23–26], both of which are risk factors for suicidal behaviour [6]. Furthermore, altered BDNF levels have been linked to suicidal behaviour in postmortem brain studies [27, 28]. Clinical studies have shown reduced peripheral BDNF levels in both the serum and plasma of suicidal individuals [29–31]. While this is a growing area of research, the relationship between BDNF levels and suicidal behaviour remains unclear, as relatively few studies have explored this relationship. In addition, since some of these studies have examined recent suicidal behaviour while others examined lifetime suicidal behaviour, it is uncertain whether BDNF is related to suicide in a distal or proximal manner. To date, there has not been a systematic review undertaken to summarize the literature.

This paper aims to systematically evaluate and summarize the existing literature relating BDNF levels (including central and peripheral levels) to suicidal behaviour (including completed suicide, attempted suicide, and suicidal ideation) in adult populations. Based on our current understanding of BDNF and its role in brain structure and function, it is expected that low BDNF levels will be associated with suicidal behaviour in studies of both central and peripheral BDNF levels.

Methods

Search strategy

The protocol for this systematic review was published previously [32]. This systematic review follows the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines as well as the Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines (see Additional file 1 for PRISMA checklist). An a priori-defined search strategy was developed with the help of an experienced health sciences librarian (LB) and was used to search the following databases from inception until December 2015: PubMed/MEDLINE, PsychINFO, EMBASE, and CINAHL. The search strategy can be found in the published protocol. One amendment was made to the original search strategy. Because many cases of suicidal behaviour involve overdose of substances, two search terms ("self poison" and "overdose") were added to capture studies of those behaviours. An example of the search strategy for MEDLINE is presented in Table 1. The grey literature was searched for previously published theses using the ProQuest Dissertations and Theses A&I database. The reference lists of included articles were scanned manually.

Table 1 Medline Search Strategy

Database	Search Strategy
MEDLINE (n=124)	1. exp Suicide/ 2. suicid*.mp. 3. exp Self-Injurious Behavior/ 4. (self harm* or self inflict* or self injur* or self wound* or self mutilat* or self poison* or overdose).mp. 5. automutilat*.mp 6. 1 or 2 or 3 or 4 or 5 7. brain derived neurotrophic factor.mp. or Brain-Derived Neurotrophic Factor/ 8. bdnf.mp 9. 7 or 8 10. 6 and 9

Inclusion and exclusion criteria

This review included observational studies that investigated associations between levels of BDNF (central or peripheral, including postmortem brain tissue, cerebral spinal fluid, and blood) and suicidal behaviours (including completed suicide, attempted suicide, and suicidal ideation) in adult populations (aged 18 and older). Studies of both clinical and community-based samples were included. No demographic limitations were applied apart from age.

Screening and data extraction

Two raters (RE and SP) screened the articles identified by the literature search. Articles were screened first by title, then by abstract for full-text review. Studies that met the inclusion criteria upon full-text review were identified for data extraction. For articles that were excluded, reasons for exclusion were documented (see Fig. 1). Discrepancies at any point in the screening process were resolved by discussion, and in cases of disagreement, a third rater (ZS) was consulted. The Kappa statistic was used to calculate inter-rater agreement [33].

Two independent raters (RE and SP) performed the data extraction in duplicate. A pre-established, pilot-tested data extraction form was used. The following information was obtained from each study: study information (title, author, publication year, journal name, location of study), study setting and design, description of sample and comparison groups, sample size, definition and measurement of suicidal behaviour, type of BDNF sample, statistical methods, mean BDNF levels and standard deviations, and limitations. Four authors were contacted for further information, and one responded with numerical data not presented in the article. One article was published in Mandarin [34], so a fluent Mandarin speaker assisted with the determination of its eligibility and data extraction. Risk of bias in included studies was assessed in duplicate using an adapted version of the Newcastle-Ottawa Scale, which assesses for risk of selection bias, performance bias, detection bias, and information bias [35].

Results

Search results

The database search retrieved 631 records. After the removal of duplicates, 488 titles were screened, and 438 records were excluded. An additional 30 were excluded upon the review of the abstracts. Twenty full-text articles were assessed for eligibility. Of these, 14 were included in this review (see Fig. 1 for PRISMA flow diagram). Inter-rater agreement for the title, abstract, and full-text screening was 0.59, 0.71, and 0.91, respectively, corresponding to fair, good, and excellent agreement [36].

Study characteristics

The characteristics of the included studies are summarized in Table 2. Twelve of the included articles were case-control studies, and two were cross-sectional studies. Four of the studies were postmortem studies that measured BDNF in brain tissue samples from individuals who had died by suicide. The remaining nine studies were clinical studies, eight of which investigated blood levels of BDNF (serum or plasma) in participants who attempted suicide, and one of which investigated cerebrospinal fluid (CSF) levels of BDNF in individuals who experienced suicidal ideation.

Risk of bias assessment

The modified version of the Newcastle-Ottawa Scale contains seven questions that fall under four domains: methods for selecting study participants (selection bias), methods to control for confounding (performance bias), statistical methods (detection bias), and methods of exposure and outcome assessment (information bias). Assessing the included studies using the modified Newcastle-Ottawa Scale revealed a number of common sources of risk of bias. Nearly all of the studies (12/14) had samples sizes that were small, likely resulting in insufficient power to detect meaningful differences in mean BDNF level between groups. Most of the studies (10/14) compared groups of between 20 and 30 participants, though some groups were as small as 10 participants. Another significant source of high risk of bias was the lack of adjustment for confounding variables. While some studies matched participants on age and/or sex in an attempt to reduce confounding, only four of the studies adjusted for any variables in their analyses. Finally, twelve of the fourteen studies used statistical methods that are inappropriate for observational studies. Univariate analyses were used to compare mean BDNF levels between groups. Only two studies [37, 38] performed a regression analysis to investigate the relationship between BDNF levels and suicidal behaviour (see Additional file 2 for a table displaying the scores from the risk of bias assessment).

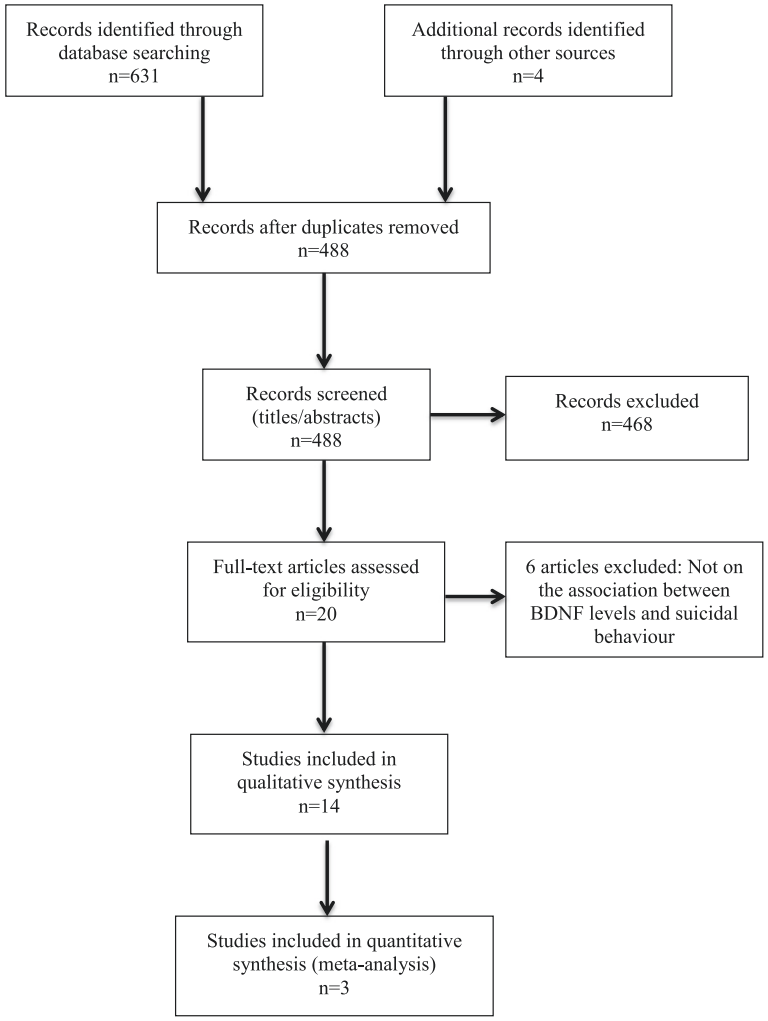

Fig. 1 PRISMA flow diagram. Studies selected for inclusion with number of studies included after each stage of the screening process

Postmortem brain studies of completed suicide

Four of the included studies examined protein levels of BDNF in the brains of postmortem subjects [27, 28, 39, 40] (see Table 2). These studies employed a case-control design to compare protein levels in individuals who died by suicide with levels in non-suicide deaths. The studies have a combined total of 90 cases and 88 controls. Dwivedi et al. (2003) [27] used the Western blot technique to determine the protein levels of BDNF in prefrontal cortex (PFC) and hippocampal samples from 27 individuals who died by suicide and 21 non-psychiatric control subjects. They found significant differences in BDNF expression between the groups in both brain regions, with lower levels in the individuals who died by suicide. The authors assert that these differences were unrelated to psychiatric diagnosis or other measured variables (postmortem interval, brain pH, age, and sex). In a similar study, Karege et al. (2005) [28] compared BDNF levels in the ventral prefrontal cortex,

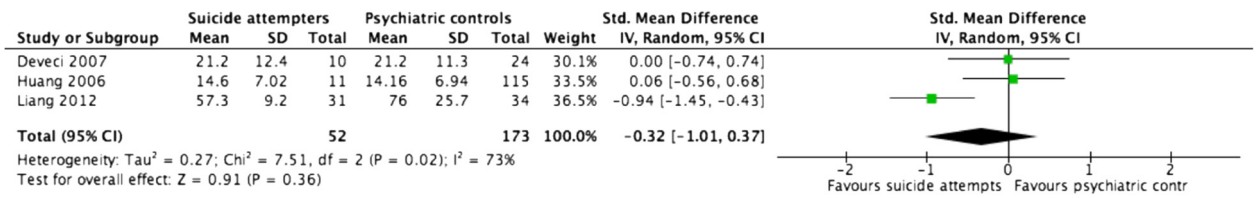

Fig. 2 Serum BDNF in attempted suicide. Legend: Forest plot representing the differences in serum BDNF level between individuals with suicide attempts and psychiatric controls

Table 2 Study Characteristics

Author (year)	Study design and sample groups	Participants (*n*)	Definition of suicidal behaviour	Type of BDNF measurement (units)	Mean (SD) BDNF levels	p-value (difference between groups)	Variables adjusted for
Postmortem Brain Studies							
Banerjee et al. (2013)	Case–control Cases of suicide vs. non-psychiatric healthy controls	Suicide: 21 Control: 19	Completed suicide	Brain – hippocampus (pg/ml)	Suicide: 19.5[a]Control: 44[a]	<0.001	None
Dwivedi et al. (2003)	Case–control Cases of suicide vs. non-psychiatric controls	Suicide: 27 Control: 21	Completed suicide	Brain – PFC, hippocampus (optical density)	PFC Suicide + MDD: 0.94 (0.22) Suicide + other psychiatric disorder: 0.88 (0.28) Control: 1.61 (0.39) Hippocampus Suicide + MDD: 1.04 (0.20) Suicide + other psychiatric disorder: 1.03 (0.22) Control: 1.71 (0.44)	<0.001<0.001	None
Karege et al. (2005)	Case–control Cases of suicide vs. non-psychiatric controls	Suicide: 30 Control: 24	Completed suicide	Brain – PFC, hippocampus, entorhinal cortex (ng/g)	Hippocampus Drug-free MDD: 17.7 (2.9) Drug-free others: 16.8 (3.1) Drug-treated MDD: 23.3 (2.2) Drug-free controls: 24.5 (3.6) PFC Drug-free MDD: 13.8 (2.6) Drug-free others: 12.7 (2.6) Drug-treated MDD: 17.9 (2.9) Drug-free controls: 17.5 (3.0) Entorhinal cortex Drug-free MDD: 14.1 (2.1) Drug-free others: 13.4 (2.4) Drug-treated MDD: 12.9 (2.3) Drug-free controls: 13.4 (2.5)	<0.001 <0.002 Not significant	PMI, age
Maheu et al. (2013)	Case–control Depressed individuals (divided into suicide victims vs. non-suicide deaths) vs. non-psychiatric controls	Depressed suicide (12) Depressed non-suicide (10) Control (14)	Completed suicide	Brain – BLA, CeA (optical density)	BLA Depressed suicide: 1.19 (0.80) Depressed non-suicide: 1.67 (1.42) Control: 1.08 (0.34) CeA Depressed suicide: 0.95 (0.20) Depressed non-suicide: 1.87 (1.30) Control: 0.97 (0.16)	Not significant Not significant	PMI, brain pH, age
CSF Studies							
Martinez et al. (2012)	Case–control Depressed patients vs. non-depressed patients	Depressed (18) Non-depressed (25)	Suicidal ideation	CSF (pg/ml)	NA	Correlation between BDNF concentration and SSI score: r = 0.62, P = 0.033, n = 12	None

Table 2 Study Characteristics *(Continued)*

Serum BDNF Studies							
Deveci et al. (2007)	Case–control Suicide attempters vs. non-suicidal MDD patients vs. healthy controls	Suicide (10) Non-suicidal MDD (24) Healthy controls (26)	Suicide attempt	Serum (ng/ml)	Suicide: 21.2 (12.24) Non-suicidal MDD: 21.2 (11.3) Control: 31.4 (8.8)	Suicide vs. healthy controls: 0.004 Suicide vs. MDD: not significant	None
Grah et al. (2014)	Case–control MDD patients vs. PD patients vs. AD patients vs. healthy controls	Suicide (96) Control (106) [b]	Suicide attempt	Serum (ng/ml)	RDD [c]Suicide: 11.8 (8.88–14.73) Control: 12.8 (10.83–14.78) PD Suicide: 10.7 (7.04–14.26) Control: 15.7 (10.29–21.02) AD Suicide: 12.6 (9.63–15.58) Control: 15.4 (12.04–18.77)	0.07 0.003 0.009	Age, sex, therapy
Huang & Lee (2006)	Case–control Schizophrenic patients vs. healthy controls	Suicidal schizophrenic (11) Non-suicidal schizophrenic (115) Healthy control (96)	Suicide attempt	Serum (ng/ml)	Suicidal schizophrenic: 14.60 (7.02) Non-suicidal schizophrenic: 14.16 (6.94)	0.841	None
Liang et al. (2012)	Case–control Depressed patients (with or without suicide attempt) vs. healthy controls	Suicidal depressed (31) Non-suicidal depressed (34) Healthy control (30)	Suicide attempt, suicidal ideation	Serum (ng/ml)	Suicidal depressed: 57.3 (9.2) Non-suicidal depressed: 76.0 (25.7) Healthy control: 113.8 (44.4)	Suicide attempt: <0.01 Suicidal ideation: <0.01	None
Park et al. (2014)	Cross-sectional MDD patients	Suicidal MDD (18) Non-suicidal MDD (33)	Suicide attempt	Serum (ng/ml)	Suicidal MDD: 21.93 (24.71) Non-suicidal MDD: 24.71 (7.7)	0.3	None
Pinheiro et al. (2014)	Cross-sectional Postpartum women	History of suicide attempts (12) No suicide history (178)	Suicide attempt, suicidal ideation	Serum (ng/ml)	Suicide: 2.11 (1.42) Control: 2.37 (1.26)	0.6 Linear regression of PPAD and suicide risk: −0.912 (−1.73– -0.09) p = 0.029	Previous psychiatric treatment, stressful life events during pregnancy
Plasma BDNF Studies							
Kim et al. (2007)	Case–control Suicidal depressed patients vs. non-suicidal depressed patients vs. healthy controls	Suicidal depressed (32) Non-suicidal depressed (32) Control (30)	Suicide attempt	Plasma (pg/ml)	Suicidal depressed: 430.5 (397.0) Non-suicidal depressed: 875.80 (663.02) Control: 889.4 (611.3)	0.002	None
Lee et al. (2007)	Case–control Depressed patients vs. healthy controls	Suicidal depressed (28) Non-suicidal depressed (49) Control (95)	Suicide attempt	Plasma (pg/ml)	Suicidal depressed: 386.61 (362.39) Non-suicidal depressed: 689.66 (404.65) Control: 819.20 (347.05)	<0.001	None
Lee & Kim (2009)	Case–control Suicidal depressed patients vs. non-suicidal depressed patients vs. healthy controls	Suicidal depressed (20) Non-suicidal depressed (20) Control (20)	Suicide attempt	Plasma [d] (pg/ml)	Suicidal depressed: 713.04 (236.56) Non-suicidal depressed: 693.98 (347.84) Control: 709.05 (172.12)	0.971	None

[a]Mean BDNF values estimated from inspection of graph
[b]3 diagnosis groups: Recurrent depressive disorder, personality disorder, adjustment disorder
[c]Medians and interquartile ranges presented instead of means and SD
[d]BDNF was measured in 3 types of sample: platelet-rich plasma, platelet-poor plasma, and platelets. Results are presented here for only the platelet-poor plasma measurement
Abbreviations: *BDNF* brain-derived neurotrophic factor, *SD* standard deviation, *MDD* major depressive disorder, *PFC* prefrontal cortex, *PMI* postmortem interval, *BLA* basolateral amygdala, *CeA* central nucleus of the amygdala, *RDD* recurrent depressive disorder, *PD* personality disorder, *AD* adjustment disorder, *PPAD* postpartum affective disorder, *CSF* cerebrospinal fluid

hippocampus, and entorhinal cortex between 30 individuals who died by suicide and 24 non-psychiatric controls. The group of individuals who died by suicide was subdivided into three groups by diagnosis and toxicology: untreated depressed, untreated other psychiatric disorder, and drug-treated depressed. The enzyme-linked immunosorbent assay (ELISA) technique was used to quantify BDNF in the tissue samples. Significantly reduced BDNF levels were found in both of the non-treated suicide groups compared to the non-suicide group, in the PFC ($p < 0.002$) and hippocampus ($p < 0.001$), but not in the drug-treated suicide group. No significant differences were found in the entorhinal cortex of any group. The third study to look at postmortem hippocampal levels of BDNF was conducted by Banerjee et al. (2013) [39]. They also employed the ELISA method to compare BDNF levels between 21 individuals who died by suicide and 19 non-psychiatric controls. A significant difference was found between the groups, with reduced BDNF levels in individuals who died by suicide ($p < 0.001$). The final study to examine brain levels of BDNF focused exclusively on the amygdala. Maheu et al. (2013) [40] measured BDNF levels in the basolateral amygdala (BLA) and central amygdala (CeA) of depressed individuals (22 and 25, respectively), and 14 healthy controls. Eleven of the depressed subjects from which the BLA was sampled died of suicide, and twelve of the depressed subjects from which the CeA was sampled died of suicide. No significant differences were found between mean BDNF levels in individuals who died by suicide compared to controls.

Cerebrospinal fluid BDNF levels and attempted suicide

Only one study examined the association between BDNF in the CSF and suicidal behaviour. Martinez et al. [41] compared levels of pro-inflammatory and "resiliency" proteins (among them BDNF) between 18 depressed individuals and 25 healthy controls. While the mean BDNF levels were not presented or compared between suicidal and non-suicidal groups, the correlation between BDNF concentration and score on the Scale for Suicidal Ideation (SSI) was calculated for 12 participants. A significant positive correlation was found between BDNF concentration and SSI score ($r = 0.62$, $p = 0.033$).

Serum BDNF levels and attempted suicide

Two of the included studies were cross-sectional studies that investigated serum levels of BDNF in clinical sample populations. These studies collectively assessed 241 individuals. Park et al. (2014) [42] conducted a pilot study relating serum BDNF levels to illness severity, suicide attempts, and central serotonin activity in depressed patients. The patients were stratified into subgroups based on their history of suicide attempts; 18 had a history of suicide attempts and 33 did not. Mean BDNF levels did not differ significantly between the two groups ($p = 0.3$). The other cross-sectional study was conducted by Pinheiro et al. (2012) [38] in postpartum women. Of the 190 women included, 12 had a history of suicide attempts. No significant difference was found between mean BDNF levels in this group compared to the women with no history of suicide attempt ($p = 0.6$). However, in women with postpartum affective disorder ($n = 29$), suicide risk, as measured with the suicidality section of the Mini International Neuropsychiatric Interview (MINI), was significantly associated with lower BDNF levels ($p = 0.02$).

The remaining four studies of serum BDNF and suicidal behaviour were case-control in design. The studies include a combined total of 148 cases and 335 controls. Two studies [29, 34] compared individuals with suicide attempts to both psychiatric and healthy controls. Deveci et al. (2007) [29] recruited 10 individuals with suicide attempts, 24 non-suicidal depressed individuals, and 26 healthy controls. Serum BDNF levels were found to be significantly lower in both the suicide group and the depressed group compared to the healthy control group ($p = 0.004$). However, there was no significant difference between BDNF levels in the suicide and depressed groups. Liang et al. (2012) [34] conducted a study comparing BDNF levels in depressed patients, with and without a history of suicide attempts, and healthy controls. The sample consisted of 31 depressed individuals with suicide attempts, 34 depressed individuals without suicide attempts, and 30 healthy controls. Serum BDNF levels were significantly different among the three groups, with the lowest levels in the suicide group ($p < 0.01$). Among the 65 depressed individuals, BDNF levels were negatively correlated with scores on the Self-rating Idea of Suicide Scale (SIOSS) ($p < 0.01$) [34].

The final two studies of serum BDNF levels focused on specific psychiatric disorders. Huang and Lee (2006) [43] measured BDNF levels in a group of 126 patients with schizophrenia, 11 of which had a history of suicide attempts. No significant difference in mean BDNF level was found ($p = 0.841$). In a study by Grah et al. (2014) [37], associations between BDNF levels and suicidal behaviour were explored in patients suffering from depression, personality disorders, and adjustment disorders. The study included 51 patients with recurrent depressive disorder, 26 of which were suicidal; 59 patients with personality disorders, 33 of which were suicidal, 62 patients with adjustment disorders, 37 of which were suicidal; and 60 healthy controls. Significantly lower BDNF levels were found in those with suicide attempts in the personality disorder and adjustment disorder groups ($p = 0.003$, $p = 0.009$, respectively), but not in the depressed group.

A meta-analysis was performed using the results of three case-control studies that compared serum BDNF levels between suicide attempters and psychiatric controls [29, 34, 43] (Figure 2). These studies were selected for inclusion in the meta-analysis based on their similar study designs (all case-control studies), definitions of suicidal behaviour (attempted suicide), and comparison groups (psychiatric controls). A random-effects model was used. The pooled estimate revealed a standardized mean difference (SMD) of −0.32 (95 % CI −1.01 to 0.37), which corresponds to a small effect size according to Cohen's criteria [44]. However, this estimate was not significant ($p = 0.36$) and was associated with substantial heterogeneity ($I^2 = 73$ %, $p = 0.02$).

Plasma BDNF levels and attempted suicide

Three case-control studies measured plasma levels of BDNF in depressed individuals with and without a history of suicidal behaviour [30, 31, 45]. These studies collectively assessed 80 cases and 246 controls. Kim et al. (2007) [30] compared 32 depressed patients hospitalized for recent suicide attempts to 32 hospitalized non-suicidal depressed patients and 30 healthy controls. They found significantly reduced plasma BDNF levels in suicide attempters compared to both control groups ($p = 0.009$, $p = 0.008$, respectively). Lee et al. (2007) [31] measured plasma BDNF levels in 77 hospitalized depressed patients (subdivided into 28 with a suicide attempt and 49 without a suicide attempt) and 95 healthy controls. This study also found a significant difference between BDNF levels in suicidal vs. non-suicidal individuals, with lower levels in the suicidal depressed group compared to the non-suicidal depressed group. Lee and Kim (2009) [45] conducted a study similar to Kim et al.'s [30] in which 20 hospitalized depressed individuals with recent suicide attempts were compared to 20 hospitalized non-suicidal depressed patients and 20 healthy controls. BDNF was measured in platelet-rich plasma, platelet-poor plasma, and platelets. In all three types of sample, BDNF levels were significantly lower in depressed patients (suicidal and non-suicidal) compared to healthy controls, but no significant differences were found between suicidal and non-suicidal groups.

Discussion

This systematic review aimed to evaluate and summarize the existing literature on associations between BDNF levels and suicidal behaviour. The 14 studies included in this review describe comparisons of mean BDNF levels between groups of individuals with and without suicidal behaviour (see Table 2). The definitions of suicidal behaviour, the methods of measuring BDNF level, and the sample populations, vary widely. The studies differ in their findings and methodological quality, producing an unclear picture of the relationship between BDNF levels and suicidal behaviour.

Postmortem brain studies of completed suicide

The postmortem studies of BDNF levels and completed suicide have examined multiple brain regions, including the hippocampus, prefrontal cortex, entorhinal cortex, and amygdala. Three studies [27, 28, 39] measured BDNF protein levels in the hippocampus and all found significant associations with completed suicide, suggesting that individuals who die by suicide have lower levels of BDNF. Two of those studies [27, 28] also measured BDNF levels in the PFC and found significant inverse associations with completed suicide. In the other brain regions studied, the entorhinal cortex [28] and the amygdala [40], no significant differences were found.

Of the four studies of brain BDNF levels in people who died by suicide, only one, Maheu et al. [40], included both psychiatric and non-psychiatric controls. The other three studies compared individuals who died by suicide to non-psychiatric controls. BDNF levels are altered in depression and other psychiatric disorders. In addition, most suicides occur in the context of a psychiatric disorder, suggesting that individuals with a psychiatric illness are a particularly vulnerable population for suicidal behaviour. In order to determine the association between BDNF and suicidal behaviour, a comparison group should be derived from a psychiatric population, in addition to healthy controls. Maheu et al.'s study was the only postmortem study that did not find a significant association between BDNF and suicide. The differences found in the other three studies could have resulted from altered BDNF levels associated with psychiatric disorders rather than suicidal behaviour. Therefore, one should be cautious when interpreting the results of the other studies, as their significant findings may not represent an association between BDNF and suicide.

Another important factor to consider is the effect of psychotropic medications on BDNF levels. Only one study, Karege et al. [28], explored this variable. They separated the group of people who died by suicide by toxicology by determining the presence of therapeutic drugs in the body. They found differences in BDNF levels among the groups. They found a significant association between BDNF level and suicidal behaviour when comparing drug-free suicide completers to controls, but not when comparing drug-treated suicide completers to controls. Future studies should investigate and control for the effects of antidepressants and other medications on BDNF levels in postmortem suicide deaths.

Postmortem studies are subject to a number of limitations, making it difficult to draw sound conclusions from them. Factors prior to death can affect the integrity of

the brain's morphology and biochemical content [46]. Depending on the cause and manner of death, changes in blood oxygenation, brain perfusion, and acid-base balance can have varying effects on the brain and on the variables of interest in postmortem studies. Different methods of suicide can produce different effects on the brains. Postmortem interval (PMI), the time between death and freezing or fixing of the brain tissue, also influences the quality of the tissue. PMI can have complex and unknown effects on the outcome measure being studied [46]. Only two of the postmortem studies of BDNF and suicide adjusted for confounding variables in their analyses [28, 40]. Both Maheu et al. and Karege et al. adjusted for PMI and age, and Maheu et al. also adjusted for brain pH. Future postmortem studies should assess and control for factors that influence the integrity of the brain tissue samples.

Bearing in mind these limitations, one can cautiously conclude from the existing evidence that an association may exist between brain levels of BDNF (particularly in the hippocampus and prefrontal cortex) and completed suicide. However, additional studies with larger samples and psychiatric comparators are needed to confirm this association.

Cerebrospinal fluid BDNF levels and attempted suicide

The one study of CSF levels of BDNF and suicidal behaviour, by Martinez et al. [41], found that increased levels of BDNF were significantly associated with higher levels of suicidal ideation. This finding is contradictory to the hypothesis that lower levels of BDNF are associated with suicidal behaviour. However, the sample size for the analysis was very small (12 participants), and the analysis did not adjust for confounding factors. Additional well-powered studies are necessary to explore this association. At this point, no conclusions can be drawn regarding the association of CSF levels of BDNF and suicidal behaviour.

Serum BDNF levels and attempted suicide

The six studies of serum BDNF levels and suicidal behaviour vary widely in their findings. Of the studies that looked at attempted suicide, three found significant associations and three did not. Two of the studies also investigated suicidal ideation and found a significant relationship with BDNF levels.

The limitations of the studies' methodologies could have resulted in biased estimates and inconsistent findings. The sample sizes were generally modest, with case groups ranging from 10 to 31 participants. None of the studies adjusted for confounding variables in their analyses, even though observational studies are inherently prone to influences by many confounding variables. Of the six studies of serum BDNF and suicidal behaviour, only two performed adjusted analyses. Grah et al. [37] adjusted for age, sex, and therapy, while Pinheiro et al. [38] adjusted for previous psychiatric treatment and stressful life events during pregnancy.

Another factor that could account for the inconsistent findings among studies is the variation in time periods between suicide attempts and BDNF measurement. While Deveci et al.'s study included individuals who were hospitalized for a recent suicide attempt, other studies included individuals with a lifetime history of suicide attempts. In studies including participants with a lifetime history of suicide attempts, the BDNF measurement could have occurred within weeks, months, or years of the suicide attempt, and the precise time interval is neither known nor accounted for in the analysis. Because BDNF levels vary over time in response to a number of external factors, the BDNF measurements in these studies may not represent the levels at the time of the suicide attempts. While it is unclear whether BDNF levels constitute a predisposing or precipitating risk factor for suicidal behaviour, studies should take into consideration the time intervals between attempt and BDNF measurement and aim for consistency. It is likely that associations between BDNF levels and suicidal behaviour will vary depending on when BDNF levels are assessed. Because only one of the six studies of BDNF level and attempted suicide included recent cases, no conclusions can be drawn regarding the relative strength of the association in recent as opposed to past suicide cases. Future studies should aim to measure BDNF in closer proximity to the suicide attempt in order to minimize the effects of unmeasured confounders that may be influenced by differences in time.

An additional point to consider is the varying methods of sample selection among studies. While some of the case-control studies separately recruited individuals who had made suicide attempts and compared them to non-suicidal controls [29, 34], other studies recruited individuals from a psychiatric population and retrospectively assessed their history of suicide attempts [37, 43]. Future studies should aim to separately recruit individuals who had attempted suicide and non-suicidal psychiatric controls in order to attain larger samples of individuals with suicide attempts and to increase the generalizability of the findings beyond individuals with a specific psychiatric disorder.

The meta-analysis of case-control studies of serum BDNF in individuals with suicide attempts and psychiatric controls revealed a small effect size of −0.32. The p value was not significant ($p = 0.36$). The high heterogeneity associated with this pooled estimate could be attributed to the diversity in the sample populations. Liang et al.'s sample consisted of patients with major depression, Huang and Lee's sample consisted of patients with schizophrenia, and Deveci et al.'s sample consisted of individuals with

suicide attempts with no major psychiatric disorder and control participants with major depression. This meta-analysis may be underpowered due to the small number of studies included and the low sample sizes in each study. Nonetheless, this is an important finding, as it suggests that individuals who attempt suicide do not have significantly altered serum BDNF levels compared to psychiatric controls.

Further research is necessary to elucidate the relationship between serum BDNF levels and suicidal behaviour, and to ascertain whether the relationship depends on the timing of measurements. Consistent definitions of suicidal behaviour, research methodology, and adjustment for important confounding factors (such as medication use, body mass index, and smoking status [18, 47, 48]) may help to produce a clearer understanding of the relationship. Currently, the evidence does not provide convincing support for an independent association between serum BDNF levels and suicidality.

Plasma BDNF levels and attempted suicide

The three studies of plasma BDNF levels and suicidal behaviour present conflicting evidence of the relationship. Two of the three studies [30, 31] found significant associations between plasma BDNF levels and attempted suicide, while the third [45] did not. It is interesting to note that two studies with very similar study designs [30, 45], in which patients with depression who were hospitalized for recent suicide attempts were compared to hospitalized non-suicidal patients with depression and healthy controls, had opposing findings. Kim et al.'s 2007 study found significantly low BDNF levels in suicidal individuals compared to both control groups, but Lee and Kim's study in 2009 found no relationship between BDNF and suicidal behaviour. The inconsistency in findings could be due to a number of factors. In all three of these studies, univariate analyses were used to compare BDNF levels among groups. While participants were matched on some variables (age and sex), no variables were adjusted for in the analyses. In addition, the sample sizes of these three studies are small; the group of individuals with suicide attempts varied from 20 to 32 individuals. Future studies should be conducted using larger samples, and using statistical analyses that adjust for confounding variables such as medication use, body mass index, and smoking status [18, 47, 48].

Another consideration is that, like in the studies of serum BDNF levels, these studies vary in the time periods between BDNF measurement and suicide attempt. Both Kim et al.'s and Lee and Kim's studies included individuals hospitalized for recent suicide attempts, while Lee's study included individuals with a lifetime history of suicide attempts. However, this does not explain the differences in findings, since the inclusion of recent vs. past suicide cases did not determine whether a significant association was found between BDNF level and suicidal behaviour.

Seeing that these three studies were all conducted at a single research centre in Korea, and may not have included independent samples, additional studies conducted in other locations with diverse sample populations will contribute valuably to the literature.

As of yet, the studies of plasma BDNF levels and suicidal behaviour are few in number, inconsistent in their findings, and subject to methodological limitations. No conclusions can be drawn from the existing evidence on the association between plasma levels of BDNF and attempted suicide.

GRADE quality of evidence

While the protocol for this systematic review stated that the Grading of Recommendations, Assessment, and Evaluation (GRADE) framework would be used to report the quality of evidence, it was deemed unnecessary to do so. The GRADE framework provides a systematic approach to consider and report risk of bias, imprecision, inconsistency, indirectness of study results, and publication bias. The GRADE framework is used to summarize and evaluate the evidence according to outcome, and is useful when the results of the studies have been combined statistically. Seeing as only 3 of the 14 included studies were pooled in a meta-analysis, it was not possible to evaluate the quality of the evidence using this framework. Furthermore, the GRADE framework is best suited to summaries of randomized controlled trials and is rarely used for observational studies such as these.

Conclusions

This is the first systematic review to explore associations between BDNF levels and suicidal behaviour. The meta-analysis of studies examining serum BDNF levels and attempted suicide revealed no significant association. The qualitative review of the literature revealed that the current evidence does not provide consistent support for an association between BDNF and suicidal behaviour. The findings of this systematic review are not in accordance with the hypothesis that lower levels of BDNF are linked to suicidal behaviour. It is possible than an association exists in parts of the brain and bloodstream, but the studies vary substantially in their methods and results, making it difficult to draw sound conclusions. The studies are also subject to a number of methodological limitations. As of yet, the studies conducted are few in number and have high risk of bias. Moreover, distinguishing the role of BDNF in suicidal behaviour from its role in mental illness is a key difficulty across studies. As this is a relatively new area of research, currently the evidence does not warrant using measures of BDNF in a

clinical setting to assess suicide risk. Further studies that are well-powered, include psychiatric comparator groups, and adjust for important confounders will help to elucidate this relationship and may provide valuable information to clinicians and researchers.

Additional files

Additional file 1: PRISMA checklist. (DOC 62.0 kb)
Additional file 2: Risk of bias assessment table. (DOCX 14.4 kb)

Abbreviations

BDNF: brain-derived neurotrophic factor; CSF: cerebrospinal fluid; MINI: Mini International Neuropsychiatric Interview; NOS: Newcastle-Ottawa Scale; SMD: standardized mean difference; SSI: Scale for Suicidal Ideation; PMI: Postmortem interval.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

RE conceived and designed the study, interpreted and analyzed the data, and wrote and revised the manuscript. SP participated in the methodology and manuscript writing. LB participated in the development of the search strategy. RA and LM critically revised the manuscript. ZS conceived and designed the study, participated in the methodology, and critically revised the manuscript. All authors read and approved the final manuscript.

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