Food Insecurity and HIV Related Health Outcomes in HIV-Hepatitis C Virus (HCV) Co-infected Individuals

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August 2017

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Doctor of Philosophy (PhD)

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
ACTG	AIDS Clinical Trials Group
CAD	Canadian Dollars
cART	Combined Antiretroviral treatment
CCC	Canadian Co-infection Cohort
CCHS	Canadian Community Health Survey
CDC	Centers for Disease Control and Prevention
CES-D-10	Center for Epidemiologic Studies Depression Scale 10 item questionnaire
FI	Food Insecurity
HFIAS	Household Food Insecurity Access Scale
HFSSM	Household Food Security Survey Module
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
IDU	Injection Drug Use
IPMW	Inverse Probability of Treatment Weights
IPTW	Inverse Probability of Mediator Weights
IQR	Inter Quartile Range
MI	Multiple Imputation
MSMs	Marginal Structural Models
OR	Odds Ratio
SD	Standard Deviation
UNAIDS	Joint United Nations Program on HIV/AIDS

ABSTRACT

Food insecurity (FI) is a significant social and health problem. Among the general population, FI is known to be associated with poor self-rated health, hypertension and depression. Among people living with HIV, FI may also have HIV-related consequences, such as poor adherence to combined antiretroviral treatment (cART), poor viral control, and poor immune recovery. Despite the evidence of adverse health impacts of FI among HIV infected people, little is known about the HIV-related health outcomes of FI among HIV-hepatitis C virus (HCV) co-infected people. The HIV-HCV co-infected people have some unique characteristics, including higher rates of injection drug use (IDU) and liver related morbidity and mortality. Therefore, findings from HIV mono-infected people may not be generalizable to HIV-HCV co-infected persons.

The overall goal of this work is to assess the association between FI and both HIV viral load and CD4 count among people co-infected with HIV-HCV; to determine if the presence of depressive symptoms mediates the effect of FI on these outcomes; and to quantify the controlled direct effect of FI that does not pass through depressive symptoms.

First, systematic literature reviews were conducted on the associations between FI and both HIV viral load and CD4 count among people living with HIV to fully understand the impact of FI among this vulnerable population subgroup. Meta-analyzed results indicated that HIV-positive people experiencing FI were more likely to have a detectable HIV viral load and a lower CD4 count compared to people who were food secure.

Second, the association between FI, and both HIV viral load and CD4 count among HIV-HCV co-infected people was assessed using data from the Food Security & HIV-HCV Sub-Study of the Canadian Co-Infection Cohort. An Inverse probability weighted marginal structural model was fitted to account for potential time-varying confounders. The results demonstrated that FI was associated with both detectable HIV viral load and lower CD4 count among co-infected people.

Third, we evaluated if the presence of depressive symptoms mediates the effect of FI on HIV viral load and CD4 count through two sub-steps: (1) assessing the association between FI and depressive symptoms, (2) assessing the associations between depressive symptoms, and both HIV viral load and CD4 count. In both analyses, marginal structural models were fitted to account for time-varying confounders. Findings demonstrated that the presence of depressive symptoms mediates the effect of FI on HIV viral load.

Lastly, a stochastic mediation analysis was conducted to assess the mediating role of depressive symptoms in the association between FI, and both HIV viral load and CD4 count. For comparison purposes, the mediating role of cART adherence was also assessed for the same associations. Results showed that both depressive symptoms and cART adherence partially mediate the effect of FI on HIV related health outcomes, indicating the existence of pathways other than depressive symptoms and cART adherence. Therefore, intervention on depressive symptoms and cART adherence was not sufficient to eliminate the harmful effect of FI on HIV viral load and CD4 count.

Through this work, we identified an important risk factor for having detectable HIV viral load and lower CD4 count among people co-infected with HIV-HCV. These patients tend to have higher mortality due to high rates of liver related death. Since unsuppressed HIV viral load and low CD4 count represent independent risk factors for liver related morbidities, addressing FI could improve both HIV related and HCV related health outcomes. Lastly, mediation analyses showed that intervention on mediators was not sufficient to block the detrimental effect of FI on HIV related health outcomes. Given the significant amount of direct effect, intervention on FI must be accompanied to improve HIV related treatment outcomes among this population subgroup.

ABRÉGÉ

L'insécurité alimentaire (IA) est un problème social et de santé important. Malgré les signes d'effets néfastes sur la santé de l'IA chez les personnes vivant avec le VIH, on sait peu de choses au sujet des conséquences de l'IA liées à l'infection par le VIH sur la santé des personnes co-infectées par le VIH-hépatite C (VHC). La population co-infectée par le VIH-VHC présente des caractéristiques uniques, dont des taux élevés de consommation de drogues injectables (UDI) ainsi qu'une morbidité et une mortalité plus élevées liées aux problèmes hépatiques.

L'objectif général de ce travail est d'évaluer l'association entre l'IA, la charge virale du VIH et la numération de cellules CD4 chez les personnes co-infectées par le VIH-VHC, de déterminer si la présence de symptômes dépressifs exerce un effet médiateur sur ces résultats, et de quantifier l'effet direct contrôlé (*controlled direct effect*) de l'IA ne passant pas par les symptômes dépressifs.

Premièrement, des revues systématiques de la littérature ont été menées sur l'association entre l'IA, la charge virale du VIH et la numération de cellules CD4 chez les personnes vivant avec le VIH. Les résultats de la méta-analyse indiquent que les personnes accablées par l'IA étaient en effet plus susceptibles d'avoir une charge virale du VIH détectable au contrôle et une numération de cellules CD4 plus faible par rapport aux personnes qui n'étaient pas en insécurité alimentaire.

Deuxièmement, l'association entre l'IA, la charge virale du VIH et la numération de cellules CD4 chez les personnes co-infectées par le VIH-VHC a été évaluée en utilisant les données de la sous-étude « Sécurité alimentaire et VIH-HCV» de la Cohorte Canadienne de Co-

infection (CCC). Un modèle structurel marginal pondéré selon la probabilité inverse a été utilisé pour tenir compte des facteurs potentiels de confusion variant dans le temps (*time-varying confounders*). Les résultats ont démontré que l'IA était fréquente chez cette population et était associée à la fois à une charge virale détectable et à une numération inférieure des cellules CD4.

Troisièmement, nous avons estimé si la présence de symptômes dépressifs avait un effet médiateur sur la charge virale du VIH et la numération de cellules CD4. Les résultats ont démontré que la présence de symptômes dépressifs module (*mediate*) seulement l'effet entre l'IA et la charge virale du VIH.

Enfin, une analyse de médiation stochastique a été menée pour quantifier l'effet direct contrôlé exercé par l'IA sur la charge virale du VIH et sur le taux de CD4 en utilisant les symptômes dépressifs comme médiateur. Dans un but de comparaison, l'analyse de l'effet direct contrôlé de l'IA sur la charge virale du VIH et sur le taux de CD4 a aussi été réalisée en utilisant l'adhérence au TARc comme médiateur. Les résultats ont démontré qu'une intervention sur ces médiateurs peut atténuer l'effet néfaste de l'IA sur la charge virale du VIH et sur le taux de CD4. Cependant, l'effet ne peut être éliminé complètement par l'intervention, même en considérant une intervention hypothétique dont l'efficacité est de 100%.

Grâce à ce travail, nous avons identifié l'IA comme étant un facteur de risque important d'une charge virale du VIH détectable au contrôle et d'une numération plus faible de CD4 chez les personnes co-infectées par le VIH-VHC. Traiter les causes de l'IA peut aider à améliorer à la fois les états de santé reliés au VIH et au VHC. En outre, les analyses de médiation ont montré

d'autres cibles potentielles d’intervention clinique afin de réduire les effets nocifs de l’IA sur la charge virale du VIH et sur la numération des cellules CD4.

ACKNOWLEDGMENTS

First and foremost, I would like to express my deepest gratitude to my thesis supervisor Dr. Paul Brassard and co-supervisor Dr. Joseph Cox. Their exceptional support and mentorship enabled me to have such a meaningful journey over the years. It would not be possible for me to successfully finish this thesis work without their remarkable guidance.

I also feel privileged to have had the support of a dedicated thesis committee: Dr. Anne-Marie Hamelin and Dr. Ashley I Naimi. My gratitude goes to their insightful guidance, comments and encouragement. Their mentorship enriched my research experience and widened my understandings of both substantive and methodological issues.

I am also grateful to Dr. Marina Klein for generously allowing me to use data from the CCC study. My sincere gratitude also goes to Dr. Erica Moodie. She provided insightful comments and suggestions throughout this thesis work.

I would like to thank everyone in the Department of Epidemiology, Biostatistics and Occupational Health. I will remember each day I spent at Purvis Hall because you all collectively made it such a welcoming place for me.

Over the past 4 years, I enjoyed so much support and friendship from classmates and fellow students: Taylor McLinden, Hiroshi Mamiya, Catherine Arsenault, Robin Richardson, Bruno Riverin, Kathryn Morrison, Laurence Brunet, Jacqueline Cohen, Ryan Kyle and others.

I am also grateful to all researchers and study coordinators at the CCC sites across Canada. My special thanks to all study participants. I would not be able to conduct my research without their active participation.

I am deeply grateful to my parents and siblings. Your encouragement, emotional and financial support enabled me to fully concentrate on my research. I will always remember your support and help. I would also like to thank my parents-in-law for their understanding and patience.

Finally, and most importantly, I am thankful to my wife, Maerziya and my daughter, Sajida. You have always been next to me during difficult times. You gave me endless joy during this journey, and your inspiration and encouragement helped me so much in pursuing my dream.

STATEMENT OF FINANCIAL SUPPORT

I would like to acknowledge the following financial support that I received during my PhD program over the past 4 years.

1. Grad Excellence Award from the department of Epidemiology, Biostatistics and Occupational Health, McGill University.

2. Graduate Research Enhancement and Travel Award (GREAT Award) for a conference in Winnipeg, Canada.

3. Research grant awarded to my supervisor Dr. Paul Brassard from the Réseau SIDA-Maladies Infectieuses of the Fond de recherche du Québec- Santé.

4. Research Grants awarded to my co-supervisor, Dr. Joseph Cox, and Dr. Marina Klein, from the Canadian Institutes of Health Research to conduct the Food Security & HIV-HCV Sub-Study and the Canadian Coinfection Cohort, respectively.

STATEMENT OF ORIGINALITY

All six manuscripts presented in this thesis constitute original contributions in the field of substantive knowledge. In addition, the method explored in the last manuscript advances the knowledge base in the field of stochastic mediation analysis.

Manuscript 1 and 2 were the first published systematic reviews and meta-analyses on the associations between food insecurity, and both HIV viral load and CD4 count, among people living with HIV. Although number of studies investigated this association previously, the results were largely inconsistent. Therefore, health professionals may need to have robust answer regarding the impact of food insecurity on HIV viral load and CD4 count. These two manuscripts systematically reviewed prior literature and determined that experiencing food insecurity does indeed have detrimental impact on both HIV viral load and CD4 count.

In manuscript 3, associations between food insecurity, and both HIV viral load and CD4 count, were assessed among people co-infected with HIV-HCV. Although prior studies assessed this association among HIV infected people with an unknown proportion of HCV co-infection, no studies assessed this association exclusively among people co-infected with HIV-HCV. Furthermore, previous cohort studies did not appear to adjust for potential time-varying confounders using appropriate methodologies. As the data in this analysis came from a cohort study involving repeated measures, it is probable that the associations are confounded by time-varying factors. Therefore, an inverse probability weighted marginal structural model was used to account for potential time-varying confounders. This is the first study that used such a novel

statistical method and assessed these associations exclusively among HIV-HCV co-infected population.

One of the objectives was to assess if depressive symptoms lie on the pathway between food insecurity, and both HIV viral load and CD4 count. This was carried out in two sub-steps using the same methodology as in manuscript 3 and produced two separate manuscripts (manuscript 4 & 5).

Manuscript 4 reported the association between food insecurity and the presence of depressive symptoms. Again, this was the first study assessing this association among people co-infected with HIV-HCV. In addition, a causal mediation analysis was conducted to assess the duration of effect of food insecurity on depressive symptoms. The result indicated that the impact of food insecurity on depressive symptoms persisted for 6-12 months even if people had recovered from food insecurity status at the current visit.

In manuscript 5, the associations between depressive symptoms, and both HIV viral load and CD4 count, were assessed using the same methods described above. There was no prior study assessing this association exclusively among HIV-HCV co-infected people. Therefore, this manuscript contributed to a current knowledge gap.

In manuscript 6, a stochastic mediation analysis with outcome redistribution approach was used to assess the mediating role of depressive symptoms in the association between food insecurity, and both HIV viral load and CD4 count. From a public health and clinical intervention perspective, it is always useful to identify potential pathways through which an exposure exerts its impact. Although the causal mediation analysis is specifically designed to identify alternative

targets for intervention, this approach may not reflect real-world scenario as it implicitly assumes the intervention on mediator is 100% effective. Stochastic mediation contrast method pioneered in mimicking real-world intervention scenario by stochastically redistributing mediator and transforming outcome accordingly. But this approach may have limitations in the presence of time-varying mediator-outcome confounders. In this manuscript, an alternative approach was explored that was built upon the marginal structural model and the stochastic mediation contrast method. Specifically, a marginal structural model is fitted to obtain mediator contribution to outcome distribution; and then both mediator and outcome are redistributed; finally, controlled direct effect of exposure is obtained using redistributed outcome variable. While the result of this mediation analysis contributed to identify mechanisms for the association between food insecurity, and both HIV viral load and CD4 count, the method used in this manuscript may contribute to the field of causal mediation methodologies.

CONTRIBUTION OF AUTHORS

Manuscript 1

Wusiman Aibibula, Joseph Cox, Anne-Marie Hamelin, Hiroshi Mamiya, Marina B Klein, and Paul Brassard. Food Insecurity and Low CD4 Count Among HIV Infected People: A Systematic Review and Meta-Analysis. AIDS Care. 2016 Dec; 28(12): 1577-1585.

Wusiman Aibibula developed the protocol, conducted literature search, analyzed data and drafted the manuscript. All coauthors assisted in editing, literature screening and provided substantive expertise.

Manuscript 2

Wusiman Aibibula, Joseph Cox, Anne-Marie Hamelin, Taylor McLinden, Marina B Klein, and Paul Brassard. Association Between Food Insecurity and HIV Viral Suppression: A Systematic Review and Meta-Analysis. AIDS Behav. 2017 Mar;21(3):754-765.

Wusiman Aibibula developed the protocol, conducted literature search, analyzed data and drafted the manuscript. All coauthors assisted in editing, literature screening and provided substantive expertise.

Manuscript 3

Wusiman Aibibula, Joseph Cox, Anne-Marie Hamelin, Erica E. M. Moodie, Ashley I Naimi, Taylor McLinden, Marina B Klein, and Paul Brassard. Food Insecurity May Lead to Incomplete HIV Viral

Suppression and Less Immune Reconstitution Among HIV-HCV Co-Infected People. Accepted HIV Medicine (September 2017).

Wusiman Aibibula developed the topic and objective of this manuscript, conducted the statistical analysis and drafted the manuscript. Paul Brassard and Joseph Cox contributed to the design of this study and provided input regarding the substantive area. Erica E. M. Moodie and Ashley I Naimi contributed to the statistical analysis section by providing guidance. Anne-Marie Hamelin and Marina B Klein contributed to the background and discussion section by providing guidance regarding the substantive area. Taylor McLinden assisted in editing the manuscript.

Manuscript 4

Wusiman Aibibula, Joseph Cox, Anne-Marie Hamelin, Erica E. M. Moodie, Ashley I Naimi, Taylor McLinden, Marina B Klein, and Paul Brassard. Impact of Food Insecurity on Depressive Symptoms among HIV-HCV Co-Infected People. Under second revision to AIDS and Behavior (September 2017).

Wusiman Aibibula developed the topic and objective of this manuscript, conducted the statistical analysis and drafted the manuscript. Paul Brassard and Joseph Cox contributed to the design of this study and provided input regarding the substantive area. Erica E. M. Moodie and Ashley I Naimi contributed to the statistical analysis section by providing guidance. Anne-Marie Hamelin and Marina B Klein contributed to the background and discussion section by providing guidance regarding the substantive area. Taylor McLinden assisted in editing the manuscript.

Manuscript 5

Wusiman Aibibula, Joseph Cox, Anne-Marie Hamelin, Erica E. M. Moodie, Aranka Anema, Marina B Klein, and Paul Brassard. Association between Depressive Symptoms, CD4 count and HIV Viral Suppression among HIV-HCV Co-Infected People. Submitted to AIDs Care (October 2017).

Wusiman Aibibula developed the topic and objective of this manuscript, conducted the statistical analysis and drafted the manuscript. Paul Brassard and Joseph Cox contributed to the design of this study and provided input regarding the substantive area. Erica E. M. Moodie contributed to the statistical analysis section by providing guidance. Anne-Marie Hamelin, Aranka Anema and Marina B Klein contributed to the background and discussion section by providing guidance regarding the substantive area.

Manuscript 6

Wusiman Aibibula, Joseph Cox, Erica E. M. Moodie, Ashley I Naimi, Anne-Marie Hamelin, Marina B Klein, and Paul Brassard. Mechanism for the Negative Impact of Food Insecurity on HIV Viral Load and CD4 Count among HIV-HCV co-infected People: Stochastic Mediation Analysis with Outcome Redistribution Approach. Submitted to Epidemiology (October 2017)

Wusiman Aibibula conceived of the method, developed the topic and objectives, conducted the statistical analysis and drafted the manuscript. Paul Brassard and Joseph Cox contributed to the design of this study and provided input regarding the substantive area. Erica E. M. Moodie and Ashley I Naimi contributed to the statistical analysis section by providing guidance. Anne-Marie Hamelin and Marina B Klein contributed to the background and discussion section by providing guidance regarding the substantive area.

Chapter 1: General Introduction

1.1 Food Insecurity

Food insecurity (FI), defined as a limited or uncertain availability of nutritionally adequate and safe foods, or limited or uncertain ability to acquire acceptable foods in socially acceptable ways [1], is a condition resulting from financial resource constraint and is usually associated with poverty [2]. According to the Food and Agriculture Organization of the United Nations, roughly one billion people worldwide were still experiencing various degrees of FI by the end of 2015 [3].

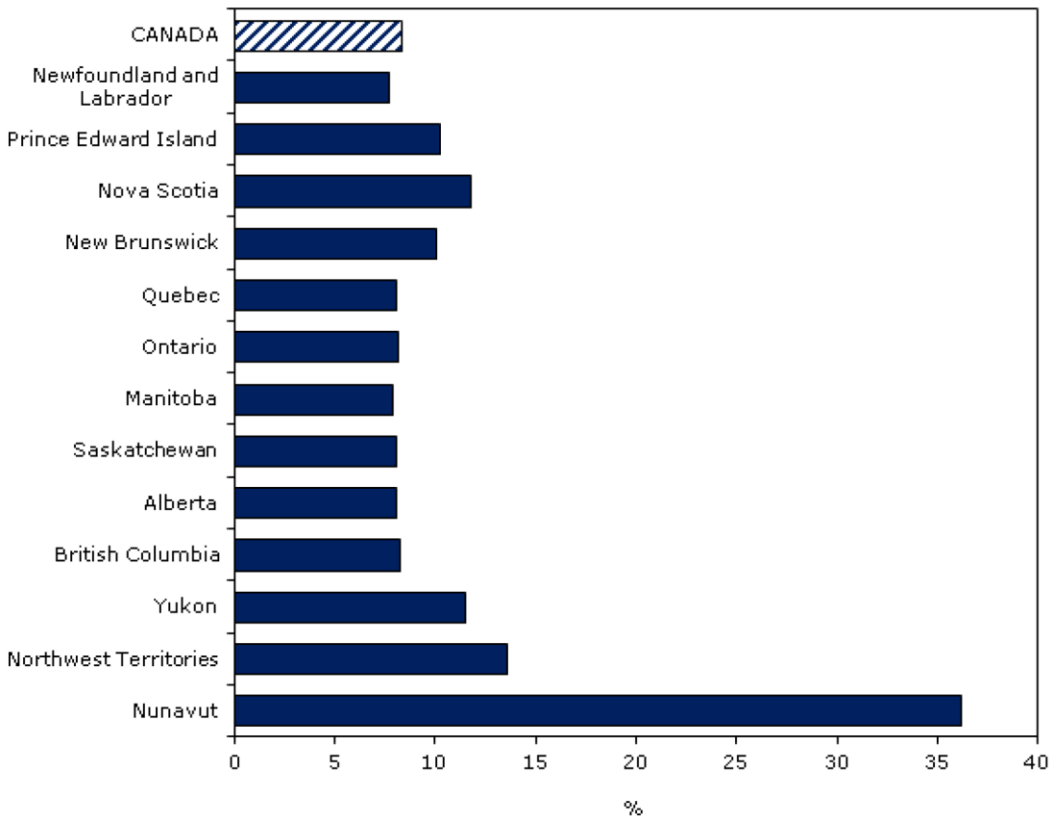
FI is a complex, multidimensional phenomenon which varies through a continuum of successive stages as the condition becomes more severe. Each stage consists of characteristic conditions and experiences of food insufficiency to fully meet the basic needs of household members, and of the behavioral responses of household members to these conditions [2]. Specifically, the first stage is psychological in nature and manifests as worrying about having enough food. The second stage involves having a certain degree of food shortage and having to reduce quality of food consumption to maintain quantity. The third stage is experienced as having more severe food shortage and having to reduce quantity of food consumption such as cutting the size of meals or skipping meals.

FI is known to be associated with various negative health outcomes in the general population, such as diabetes, psychological stress, and poor physical health [4-11]. Recognized as an important public health issue, household FI has been monitored in Canada since 2004 through national surveys, including cycles of the Canadian Community Health Survey (CCHS)

using the US Department of Agriculture's Household Food Security Survey Module (HFSSM) [2,12]. The HFSSM consists of 18 questions, 10 of them are used for measuring adult level FI and the remaining 8 are specific to the food experience of children under 18 [2]. The CCHS classifies FI based on the number of affirmative answers to the HFSSM questionnaire. Adult food insecurity status is classified into three categories: food security (0 or 1 affirmative answer), moderate FI (2-5 affirmative answers), or severe FI (≥6 affirmative answers). An answer is considered affirmative if it is either "often true" or "sometimes true" to questions such as "Since your last visit, did you or other adults in your household ever cut the size of your meals or skip meals because there wasn't enough money for food?" (Appendix A).

Based on data from the 2011-2012 CCHS, 8.3% of Canadian households, or almost 1.1 million households, experienced FI; of which 5.8% was reported as moderate FI and 2.5% as severe [13]. Figure 1.1.1 displays FI prevalence across different provinces and territories in Canada.

Figure 1.1.1 Percentage of households with food insecurity, by provinces/territory, Canada 2011-2012



Source: Canadian Community Health Survey, 2011-2012

1.2 Human Immunodeficiency Virus (HIV) Infection

The HIV is a retrovirus that infects vital cells in the human immune system such as CD4 cells. Since it replicates itself using the host cell structure, the replication is accompanied with destruction of immune cells [14]. As a result, gradual depletion of CD4 cells with increasing HIV viral load is a typical natural history of HIV infection [15]. Depletion of immune cells will lead to various opportunistic infections.

HIV infection remains a major public health challenge across the globe. There were an estimated 36.7 million people living with HIV in 2015 and an estimated 2.1 million new infections added in that year alone [16]. Eastern and Southern Africa has more than half of the

global burden of HIV infection, whereas less than 10% of HIV infected people live in Western and central Europe and North America [16]. The estimated number of Canadians living with HIV at the end of 2014 was 75,500, a 9.7% increase from 2011 [17]. This increase is mainly due to improved survival of persons infected with HIV, and new infections also continue to occur, largely in key populations, such as men who have sex with men [17].

1.3 Hepatitis C Virus (HCV) Infection

Hepatitis C Virus (HCV) is an RNA virus that causes both acute and chronic liver infection. About two third of HCV infected people become chronic, while the remaining may spontaneously clear the virus without requiring any treatment. Chronic HCV infection is a major cause of mortality and morbidity across countries, with an estimated 177 million people infected worldwide [18].

Due to similar routes of transmission, HCV infection among HIV infected people is relatively common [19], and unlike HIV negative persons, the majority of HCV infection among HIV positive people become chronic [20]. According to Hull et al [21], there were over 13000 individuals with HIV-HCV co-infection in 2008, accounting for approximately 20% of the total HIV positive people in Canada in that year. Given increased life expectancy of people living with HIV [22,23] and the chronic nature of HCV infection within this group [20], the prevalence of HIV-HCV co-infection may continue to increase.

1.4 Food Insecurity among HIV Infected people

Due to characteristics, such as HIV related morbidities, unemployment and injection drug use (IDU), FI is common among HIV infected people. Studies conducted in Canada and United States (US) have consistently reported that more than half of their respective study samples had FI [24-28]. Being FI is related to poor medication adherence [29,30], worse immunologic and virologic outcomes [28,29,31,32], increased opportunistic infections and health care utilization [33]. Furthermore, FI may also induce anxiety and detrimental coping mechanism, such as risky sexual behaviours [34,35]. As such, FI may increase HIV transmission in the community [36-38].

1.5 Depression among HIV infected people

Depression is a relatively common psychiatric disorder among HIV infected people [24]. In a nationally representative sample of HIV infected people receiving care in the US, up to one third of the study sample had a major depression disorder [39]. A Meta-analysis showed that the prevalence of depression was nearly two times higher among HIV positive people than an HIV negative comparison group [40]. Diagnosis of HIV and associated social stigma may contribute to the development of depression among HIV infected people [41]. In addition, FI is also found to be strongly associated with depression among this population [42,43]. It was also shown that depression has an array of negative health impacts including poor combined antiretroviral treatment (cART) adherence, risky sexual behavior and HIV disease progression [44-47].

Although FI and its potential health impacts have been studied among HIV infected people [4-11,28-38], no prior studies have determined whether those negative health impacts

persist among HIV-HCV co-infected people. Given the increasing number of people living with HIV-HCV co-infection and the anticipated high prevalence of FI within this population [25] assessing HIV related health impacts of FI among this population may provide valuable knowledge in terms of reducing infectious disease transmission and improving individual health status. Lastly, assessing the mediating role of depression in the association between FI and HIV related health outcomes may provide additional public health target. Specifically, if a significant amount of FI effects passes through depression, then intervention on depression may provide additional benefit in eliminating harmful effect of FI on HIV related health outcomes.

1.6 Study Goal and Objectives

The overarching goal of this project is to determine if food insecurity (FI) has any impact on HIV viral load and CD4 count among HIV-HCV co-infected people and to explore potential mechanisms for this association.

Specific objectives include:

1. To conduct systematic review and meta-analysis to understand FI and its association with HIV viral load and CD4 count among people living with HIV.

2. To estimate the association between FI, and both HIV viral load and CD4 count, among people co-infected with HIV-HCV.

3. To identify if presence of depressive symptoms mediates the effect of FI on HIV viral load and CD4 count. This is to be assessed using the following steps.

    a. Quantifying the association between FI and depressive symptoms.

b. Quantifying the association between depressive symptoms, and both HIV viral load and CD4 count.

4. To determine the magnitude of the controlled direct effect of FI on HIV viral load and CD4 count using presence of depressive symptoms as a mediator.

1.7 Organization of Thesis

This manuscript-based thesis is organized as follows. Chapter 2 provides the literature review on the associations between FI, and both HIV viral load and CD4 count, among HIV infected people where an unknown proportion are also HCV co-infected. This chapter contains two manuscripts: “Food Insecurity and Low CD4 Count among HIV Infected People: A Systematic Review and Meta-Analysis” and “Association Between Food Insecurity and HIV Viral Suppression: A Systematic Review and Meta-Analysis”. Chapter 3 assesses the overall associations between FI, and both HIV viral load and CD4 count, among people co-infected with HIV-HCV. Chapter 4 examines the association between FI and depressive symptoms among co-infected people. Chapter 5 assesses the association between depressive symptoms, and both HIV viral load and CD4 count, among co-infected people. The results of chapters 4 and 5 determine if depressive symptoms lie on the pathway between FI, and both HIV viral load and CD4 count. Chapter 6 assesses the controlled direct effect of FI on HIV viral suppression and CD4 count among HIV-HCV co-infected people. Chapter 7 provides an overall summary of the findings, related implications and concluding remarks.

Chapter 2: Literature Review

2.1 Preface to Manuscript 1 and 2

It was estimated that almost half of HIV infected individuals globally are receiving combined antiretroviral treatment (cART) [16]. Despite this marked increase in cART coverage, not all HIV infected people initiating cART achieving HIV viral control and immune recovery. Studies indicated that about 25% of those receiving cART did not achieve complete HIV viral suppression [48-50] and remained at relatively low CD4 level [51].

Many factors may contribute to these suboptimal treatment outcomes, including treatment interruptions and non-adherence [52,53] as well as late initiation of treatment [54]. Recently, FI has been identified as a potential risk factor for poor virologic response [28,31] and lower CD4 count [55] among people living with HIV. It was shown that FI may lead to poor medication adherence [56,57] and poor medication adherence is a strong predictor of poor treatment outcomes. On the other hand, FI may also exert its effect through other pathways. For example, people may reduce food consumption as they experience FI [58,59] and this may lead to nutritional deficiency over the long term [60]. Nutrition is essential if the immune system is to function properly against HIV [61,62].

Despite the plausibility for the association between FI, and both HIV viral load and CD4 count, published literature results are inconsistent and there is no prior systematic review summarised this association. To better understand HIV related health impacts of FI, we conducted two systematic reviews examining the associations between FI, and both HIV viral load and CD4 count: 1. the association between FI and low CD4 count. 2. the association

between FI and HIV viral suppression. These reviews provided support for the hypothesized associations and helped overcoming inconsistencies in the literature.

Manuscript 1 was presented at the 25th Annual Conference of the Canadian Association for HIV Research in Winnipeg, Canada, in May 2016.

Manuscript 2 was presented at the department of Epidemiology, Biostatistics and Occupational Health 2016 Research Day in April, 2016; at the 25th Annual Conference of the Canadian Association for HIV Research in Winnipeg, Canada, in May 2016; at the Canadian Society for Epidemiology and Biostatistics National Student Conference in Winnipeg, Canada, in June 2016; and at the Research Institute of the McGill University Health Centre Best of Core Research Day in Montreal, Canada, in December, 2016.

2.2 Manuscript 1

Food Insecurity and Low CD4 Count among HIV Infected People: A Systematic Review and Meta-Analysis

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Publication status: AIDS Care. 2016 Dec; 28(12): 1577-1585.

Abstract

Objectives: To systematically review published literature to determine the association between food insecurity and CD4 count among HIV infected people.

Methods: PubMed, Web of Science, ProQuest ABI/INFORM Complete, Ovid Medline and EMBASE Classic, plus bibliographies of relevant studies were systematically searched up to May, 2015, where the earliest database coverage started from 1900. Studies that quantitatively assessed the association between food insecurity and CD4 count among HIV infected people were eligible for inclusion. Study results were summarized using random effects model.

Results: A total of 2093 articles were identified through electronic database search and manual bibliographic search, of which 8 studies included in this meta-analysis. Food insecure people had 1.32 times greater odds of having lower CD4 counts compared to food secure people (OR=1.32, 95% CI 1.15-1.53) and food insecure people had on average 91 fewer CD4 cells/µl compared to their food secure counterparts (mean difference=-91.09, 95% CI -156.16, -26.02).

Conclusion: Food insecurity could be a potential barrier to immune recovery as measured by CD4 counts among HIV infected people.

Keywords: HIV; food insecurity; CD4 count; meta-analysis.

Introduction

HIV infection is known to cause the selective loss of CD4 cells due to virus replication and subsequent cell destruction [14]. Therefore, CD4 count is often used as an important measure of HIV progression and as one of the indications for treatment initiation [63]. While effective antiretroviral treatment can significantly increase CD4 counts in the majority of patients, there are certain populations who remain at relatively low CD4 count levels [51]. Factors possibly associated with poor CD4 recovery have been extensively studied. Examples of such factors include older age, ongoing HIV replication [51], treatment interruption and non-adherence to combined anti-retroviral therapy (cART) [52,53], and late initiation of treatment [54]. Since CD4 level predicts mortality [64], and low CD4 count is associated with an increased risk of opportunistic infections, it is important to investigate all possible factors that impede immune recovery among HIV infected people.

In recent years, food insecurity has been identified as an issue that is critically important for HIV infected people as it is associated with unprotected sex, suboptimal treatment adherence, incomplete viral suppression and lower CD4 counts [28,30,34,55,65]. Food insecurity is defined as a limited or uncertain ability to acquire acceptable foods in socially acceptable ways, or limited or uncertain availability of nutritionally adequate and safe foods [1]. This definition implies that food insecurity is a broader concept than only a physical shortage of food since it includes all of the psychological, physical and social aspects of reduced access to sufficient quality foods. Food insecurity represents a series of stages where the first is psychological in nature and manifests as *worrying* about having enough food. The second stage

involves *having a certain degree* of food shortage and having to reduce food quality to maintain quantity. The third stage is experienced as *having more severe* food shortage and *having to reduce food quantity* such as cutting the size of meals or skipping meals. When people experience food insecurity, different coping behaviors, such as reduced quality and quantity of food consumption [66] may make it difficult to meet minimum nutritional requirements leading to nutritional deficiencies over the long term [60]. This can lead to immune suppression, acceleration of HIV replication and subsequent CD4 depletion among HIV infected people [67]. It is also possible that having to make a choice between food and medication further exacerbate this problem. However, the association between food insecurity and lower CD4 count is inconsistent in the literature. For example, study conducted by Wang et al [31] found significant association between food insecurity and low CD4 count in unadjusted model. However, that significant association disappeared after adjusting for important confounder variables. The authors explained this result by noting that "One possible explanation for this discrepancy is that previous studies have reported that food insecure individuals had significantly lower CD4 counts prior to initiation of antiretroviral therapy, whereas our study examined the association among individuals already prescribed antiretroviral medications. As a result, the effect of food insecurity on an individual's immunologic response to HIV was less likely to be detected given the marked improvements in CD4 counts when individuals are started on antiretroviral medications" [31].

The prevalence of food insecurity among HIV infected people is consistently higher than that among the general population. For example, a study conducted in the US found that more than 63% of their study samples were food insecure on one or more occasions [55], and it was

59% in another Canadian study conducted among HIV/HCV co-infected people [25]. Therefore, it is important to understand its possible impacts on HIV related health outcomes. In this review, we aim to determine the association between food insecurity and low CD4 count among HIV infected people with an intention to overcome discrepancies in the literature.

Methods

We developed methods according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement [68].

Literature Search and Selection Criteria

We aimed to collect all relevant studies that quantitatively assessed the association between food insecurity and CD4 count or studies where this information was available, and systematically searched five electronic databases--PubMed, Web of Science, ProQuest ABI/INFORM Complete, Ovid Medline and EMBASE Classic from the date of database inception to May 15th, 2015, where the temporal coverage of Web of Science started from 1900. The initial search strategy was developed for PubMed and then adapted to the other databases. The following themes were used to locate articles: i) HIV infection: human immunodeficiency viruses OR hiv OR aids, ii) Food insecurity: food security OR food insecurity OR hunger, and iii) CD4: cd4 cell count OR cd4 cell counts. Finally, all three themes were combined using the Boolean operator AND. Bibliographies of relevant articles were manually searched. The full search strategy is provided in Appendix 2.2.1

Two reviewers (W.A. and H.M.) independently assessed all titles and abstracts and the full-text, if necessary. Studies were included according to the following inclusion criteria: (1) any

quantitative studies examining the association between food insecurity and CD4 counts (both continuous CD4 count and dichotomized CD4 level based on certain cut-off values) among HIV infected people, or if information could be obtained so as to calculate mean differences or OR. (2) peer reviewed English, French or Chinese articles. The study selection process is detailed in Figure 2.2.1.

Data Extraction and Quality Assessment

A standardized data extraction form was developed and two reviewers (W.A. and H.M.) independently extracted information on first author, publication year, design and period of study, geographic settings, sample size, adjustment factors, number of exposed and unexposed, mean age (overall and by exposure groups), response rate, loss to follow-up, sample proportion on cART and effect measures. Upon completion of data extraction, the two sets of extracted data were compared. Discrepancies were resolved through consensus. The Newcastle Ottawa Scale (NOS) [69] was used to assess study quality for cohort studies and an adapted version of the tool was used to assess the quality of cross-sectional studies (Appendix 2.2.2). Adaptation was based on work by Herzog et al [70] with slight modification so that the adapted version has nine stars maximum rather than ten stars as in original scale by Herzog et al [70]. The NOS is composed of three domains: sample selection, comparability and outcome measurement. Each domain contains several aspects that collectively support the quality of the respective domains. For example, in comparability, controlling for important confounders is an essential aspect in order to have a higher score for that domain. The overall score ranges between 0 and 9 stars; more stars representing better quality. Study qualities were cross checked and any discrepancies were also resolved through consensus.

Study Outcomes

Association measures expressed as Odds Ratio (OR) were directly used. If, however, studies only provided mean CD4 count by exposed and un-exposed groups and/or the numbers of subjects by exposure status (food insecurity status) and immune status (CD4 count below a certain cutoff), attempts were made to obtain an adjusted mean difference in continuous CD4 count or an adjusted OR by contacting study authors. When author contacts were unsuccessful, the unadjusted mean difference was calculated as the mean difference=[(mean CD4 among food insecure group)-(mean CD4 among non-food insecure group)]. Standard error calculation is detailed in Appendix 2.2.3; unadjusted OR and 95% confidence interval were calculated by constructing two by two tables. One study [55] only provided the linear regression coefficient for food insecurity: -99.52 and P<0.001. Therefore, we used the P=0.001 to be conservative and computationally feasible and calculated its standard error according to the Wald method [71] as $z = \frac{\beta_{estimate} - \beta_{null}}{estimated\ SE}$. Standard errors were used to construct forest plot. Another study [26] did not provide the mean CD4 count by food insecurity status, instead providing separate mean CD4 counts by prescribed medications that require food and do not require food within the food insecure and secure groups. In order to obtain the mean CD4 count and its standard deviation relative to exposure status (food insecurity), we combined means and standard deviations within the food insecure and secure groups, and used these combined means and standard deviations in the statistical analyses.

Statistical Analysis

Since we have two distinct association measures across studies, OR and mean difference, we pooled these results separately. Heterogeneity between studies was assessed using the Higgins I^2 test [72]. In order to allow for underlying differences in study design, study participants and study settings, a Dersimonian-Laird Random Effects meta-analysis [73] was performed to pool results across studies regardless of the significance of between studies heterogeneity. Although power is limited due to the small number of studies included, we still performed a funnel plot and meta-bias analysis to see if there was any publication bias. We specified the Egger test method for both meta-bias analysis and funnel plot construction.

Results

The electronic database search and manual search produced 1863 unduplicated citations and after a title/abstract review, 23 articles were retained for full-text review, of which 8 articles were eligible for final inclusion (Figure 2.2.1).

Study Characteristics

The final review included three cohort studies and five cross-sectional studies with total sample size of 4589 HIV infected people (Table 2.2.1). Study sample sizes ranged from 62 to 1860 (median 391; IQR 267-676). Length of follow-up reported by three prospective studies was from 22 months to 52.8 months. One study [74] was conducted among children with a mean age of 14 years old. Three studies [32,75,76] provided mean ages by exposure group and the corresponding overall ages were expressed as weighted averages. Study quality was good overall with mean total stars of six. This is related to relatively large sample sizes enabling investigators to adjust for more and important confounders achieving better internal validities.

All three longitudinal studies [55,75,76] provided baseline CD4 counts either as overall or by exposure group, and it ranged from 137 to 331 cells/µl.

Four studies [31,32,75,76] provided OR as an association measure, three of them [31,32,76] used CD4≥200 as the immune recovery cut-off and one study [75] used CD4≥350 as the immune recovery cut-off. The number of study subjects whose CD4 counts<200 among exposed (food insecure) and unexposed (food secure) groups was available in one study [26]. Therefore, we calculated the OR by constructing a two by two table and used this OR in constructing the forest plot (Figure 2.2.2) after author contact failed. One study [74] provided covariate adjusted mean CD4 count differences between exposure groups, and the mean CD4 count was available by exposure status or could be calculated in the other two studies [26,29]. One study [55] presented linear coefficient corresponding to a dichotomous exposure variable and its P value as an effect measure (this P value was used to calculate the standard error corresponding to that coefficient as stated in the methods section). Although one study [31] provided median CD4 count and Interquartile Range by exposure groups, we were able to obtain covariate adjusted mean difference and standard errors upon author contact.

Exposure and Outcome Ascertainment

Food insecurity was measured using validated instruments although three of them have used portion of the originally validated instrument and thus the original validity property may have been modified. One study [55] used the adult-level food security questions of the Radimer/Cornell Scale. This questionnaire has four questions covering qualitative, quantitative and psychological aspects of adult food insecurity [77]. Four studies [31,32,75,76] used the

Household Food Insecurity Access Scale (HFIAS) [78], and among them one study [31] used only the first item of questionnaire while the other studies used the whole scale. The first question of this instrument measures the psychological component of food insecurity, while the remaining items concern physical food shortage. Three studies [26,29,74] used the US Department of Agriculture's Household Food Security Survey Module (HFSSM) [2]. This instrument consists of 18 items, 10 of which are used to measure household level food insecurity and the remaining 8 items are used to measure child-level food insecurity in the household. In the study conducted by Mendoza et al [74], the investigators used the entire 18-item questionnaire to determine both household and child-level food insecurity; another study [26] adapted eight items of the original instrument focusing on food insecurity status over the past month period; and the last study [29] adapted six items of the original instrument to measure food sufficiency in the previous year. Except for one study [29] who used patient-reported CD4 count as the outcome variable, remaining studies used laboratory reported CD4 counts as the outcome. Study characteristics, exposure measurement and confounders adjusted for in each study are provided in Table 2.2.1.

Pooled Results

All five studies from which an OR can be retrieved showed that people with food insecurity had increased odds of lower CD4 count regardless of the CD4 cut-off used; the point estimate of ORs ranged from 1.1 to 2.08. Three studies had confidence intervals containing the null value, while the other two had confidence intervals that did not (Figure 2.2.2). The pooled estimate showed that there was a 32% increased odds for lower CD4 counts among people with food insecurity (OR=1.32, 95% CI 1.15-1.53). A subgroup analysis was also conducted to see if

this association was consistent across different study designs. Figure 2.2.2 shows that the association between food insecurity and CD4 is stronger among cohort studies (OR=1.36, 95% CI 1.15, 1.61) while this association is not significant for cross-sectional studies (OR=1.31, 95% CI 0.96, 1.78). The I^2 test showed low to moderate between study heterogeneity (Figure 2.2.2).

Results from studies who provided mean CD4 counts by exposure group are shown in Figure 2.2.3. Point estimates from all five studies showed that people with food insecurity had lower CD4 counts than food secure people although one [31] had a confidence interval that includes the null. According to the random effects model, people with food insecurity had on average, 91 fewer CD4 cells, compared to food secure people (mean difference=-91.09, 95% CI -156.16, -26.02). However, the I^2 test, which is I^2=90.4%, indicated that there was high between studies heterogeneity (Figure 2.2.3).

Publication Bias Assessment

Publication bias was assessed with funnel plot (figure not shown) and a meta-bias analysis. This analysis was conducted using studies providing mean CD4 counts first and then using studies providing ORs. Both funnel plot and meta-bias analyses did not show any indication of publication bias. (Estimated bias coefficient=0.19, SE=0.21, t=0.93, P=0.42).

Discussion

The eight studies included in this meta-analysis suggest that people living with HIV who experience food insecurity tend to have lower CD4 count compared to their food secure counterparts. Importantly, the pooled association is statistically significant among prospective cohort studies while it is not among cross-sectional studies (Figure 2.2.2). Four studies

[26,31,32,76] used CD4 cut-offs of 200, and one study [75] used a cut-off of 350. However, the study using 350 as the cut-off found the second highest OR among all five studies (Figure 2.2.2). It is possible that the other studies would have had greater OR values had they used the 350 as cut-off value; that is because it takes a relatively shorter period of time for the CD4 count to reach the 350 threshold compared to 200. Also during the study period, some participants may have had a CD4 count already below 350 but had not reached the threshold of 200. Pooled results from studies that only provided a mean CD4 count by exposure group showed that people with food insecurity had 91 fewer CD4 cells compared to food secure people (Figure 2.2.3). These results were consistent with the results of another study [79] involving a pediatric population. In that study food insecurity was expressed as a continuous HFIAS score [78] and showed that every one unit increase was associated with a 0.6% decrease of CD4 percentage.

These findings are biologically plausible given that different coping behaviors such as reduced food consumption and relying on less preferred but less expensive foods may occur when people experience food insecurity [66]. These coping behaviors may make it difficult to cover minimum nutritional requirements leading to nutritional deficiencies over the long term. Although the exact mechanism in which food insecurity is associated with lower CD4 counts is not yet fully understood, both experimental and human studies have demonstrated that adequate nutrition (sufficient intake of nutritious foods and the absorption of nutrients) is essential if the immune system is to function normally. For example, antioxidant nutrients maintain the antioxidant/oxidant balance in immune cells offering protection from oxidative stress, and preserving adequate function [80]. Furthermore, protein deficiency causes both numerical and functional deficiencies in cell immunity [81]. It has also been shown that

impaired immune function caused by nutritional deficiency can be improved through micronutrient supplementation [82].

Family rations that provide food for the HIV positive individuals can be an immediate option to improve food insecurity status among HIV infected people. This approach is widely used even in resource limited settings [83] and it has promising effect on improving cART medication adherence and subsequent health outcomes [84,85]. However, sustainable solution for food insecurity must focus on upstream causes of this problem. A recent study found that injecting drug use and depressive symptoms were significantly associated with being food insecure among HIV/HCV co-infected people in Canada and suggested that harm reduction program and mental health services may help to mitigate food insecurity among this population [25].

The critical appraisal of included studies demonstrated some minor flaws. First, Wang et al [31] used the first question of HFIAS [78] to measure food insecurity. While it is possible that this may have led to a biased prevalence estimate of food insecurity because of the limited ability of one question to capture the whole domain of food insecurity, it is unlikely that study participants were aware of the study hypothesis and systematically misreported food insecurity status according to their CD4 counts. However, non-differential exposure misclassification may diminish statistical power, and probably explains why an association between food insecurity and CD4 count was not found in that study. Second, the studies by Kalichman et al [26,29] provided mean CD4 counts and the numbers of subjects with CD4<200, according to food insecurity status. We have computed mean CD4 count differences and ORs based on the information provided. However, the computed differences of CD4 count and ORs may not

necessarily reflect the true associations between food insecurity and CD4 count. Instead, differences could be due to the confounding effects of other factors. For these reasons, the two studies received relatively low scores in quality assessment. Third, although a CD4 measurement error is possible in the study by Kalichman, et al [29] due to self-reporting, it was likely non-differential between exposed and unexposed groups. Therefore, this may have led to a small underestimation of the actual association. The pooled estimate after exclusion of this individual study was not materially different (after exclusion of this individual study, the mean difference =-91.24, 95% CI -166.84, -15.65). Fourth, study by Weiser et al [75] assessed the effect of food insecurity in a prospective cohort study. Unfortunately, authors only mentioned Generalized Estimating Equations (GEE) without specifying how they controlled time-varying confounders in their repeatedly measured cohort study. Time-varying confounding is possible in repeated measurement settings. For example, medication adherence may be affected by prior food insecurity status and also may affect food insecurity status measured at a later time point perhaps due to worsened health status caused by poor adherence. Medication adherence is also a good predictor of CD4 count; as such, it should be treated as a time-varying confounder affected by prior exposure. In this setting, the Marginal Structural Model (MSM) or Structural Nested Model (SNM) [86,87] would be more appropriate to control for time-varying confounding.

Several limitations of this meta-analysis must be noted. First, exposure measurement tools were different from one study to another and some studies have used only part of originally validated instruments (this may have discounted validity properties). This may have led to different sensitivities and specificities of the scales in detecting food insecurity, however,

exposure misclassification was likely non-differential. Second, although the bias analysis did not show signs of a publication bias, power is limited as we used only five studies to conduct the meta-bias analysis. Third, included studies have adjusted for many important confounders, such as baseline CD4 and socioeconomic status. However, it is impossible to rule out the possibility of residual confounding as in any observational study regardless of how many confounding variables were included in the analysis. For example, if there were underlying undetected health conditions, then people with that health condition would be more likely to be food insecure and also more likely to have lower CD4 counts. As a result, the low CD4 count found among food insecure people may not only be the result of food insecurity, but perhaps also the result of the underlying health condition. Fourth, we limited our search to English, French and Chinese languages, and therefore, we may have missed studies published in other languages.

Overall, the evidence from this meta-analyses showed a relatively strong association between food insecurity and lower CD4 count among HIV infected people. The data were consistent both in resource rich and limited settings and across different study designs. Therefore, addressing food insecurity among HIV infected people must be considered if we are to improve immune status and avoid poor health outcomes.

Table 2.2.1 Characteristics of included studies

Author	Study design	Study period	Country	Sample size	Age (SD)	Exposure measurement	Confounders adjusted	Quality
McMahon et al (2011) [55]	Cohort	1995-05	USA	592	40.6 (7.5)	Radimer/Cornell Scale (Individual/adult level)	Age, gender, race, cumulative years of cART at final study visit, history of IDU up to final study visit, mean BMI, poverty at final study visit.	6 stars
Wang et al (2011) [31]	Cross sectional	2002-08	USA	1860†	49.5‡ (8.9)	Used first question of 18 item HFIAS (household level)	Age, gender, race, income, education, marital status, employment, homelessness, site of enrollment, alcohol use, drug use, cART adherence.	7 stars
Kalichman et al (2015) [26]	Cross sectional	2013-14	USA	759	48‡ (8.5)	8 of 18 items HFSSM (household level)	NS	5 stars
Mendoza et al (2013) [74]	Cross sectional	2010-11	USA	62	14 (5.2)	18 item HFSSM (household level)	Age, gender, race, BMI, health insurance status, cART status, parent education level	6 stars
Weiser et al (2009) [32]	Cross sectional	2006	USA	250	46.5 (7.9)	9-item HFIAS (household level)	Age, race, income, education, recent homelessness, employment, health insurance, drug use, alcohol, smoking, sex exchange, incarceration, number of friends.	6 stars

Weiser et al (2013) [76]	Cohort	2007-10	USA	284	48 (IQR 43-54)	9-item HFIAS (household level)	Time fixed: sex, age, ethnicity, income, education, months on cART at baseline, nadir CD4 count. Time varying: recent homelessness, health insurance, drug use, alcohol, smoking, cART adherence, VL level.	8 stars
Weiser et al (2014) [75]	Cohort	2007-10	Uganda	438	35.4 (8.5)	9-item HFIAS (household level)	Time fixed: age, sex, marital status, education, cART status at baseline, Pre-cART CD4. Time varying: cART adherence, viral suppression, employment, household asset index, alcohol drinking and smoking	8 stars
Kalichman et al (2010) [29]	Cross sectional	2008-09	USA	344	44.4‡ (7.8)	6 of 18 items HFSSM (household level)	NS	3 stars

Notes and Abbreviations: Radimer/Cornell Food Insecurity scale was developed by Radimer el, al and modified by Kendall, et all (Individual/adult level); HFIAS, Household Food Insecurity Access Scale (Household level); HFSSM, Household Food Security Survey Module (Household level); (..), Not available; † indicates sample size used for multivariate model, not the total sample size in the original study; ‡ mean age and its standard deviations provided separately in original papers were combined using sample mean and standard deviation combining formulas; NS, Not stated.

Figure 2.2.1 PRISMA flow diagram for studies included in the meta-analysis

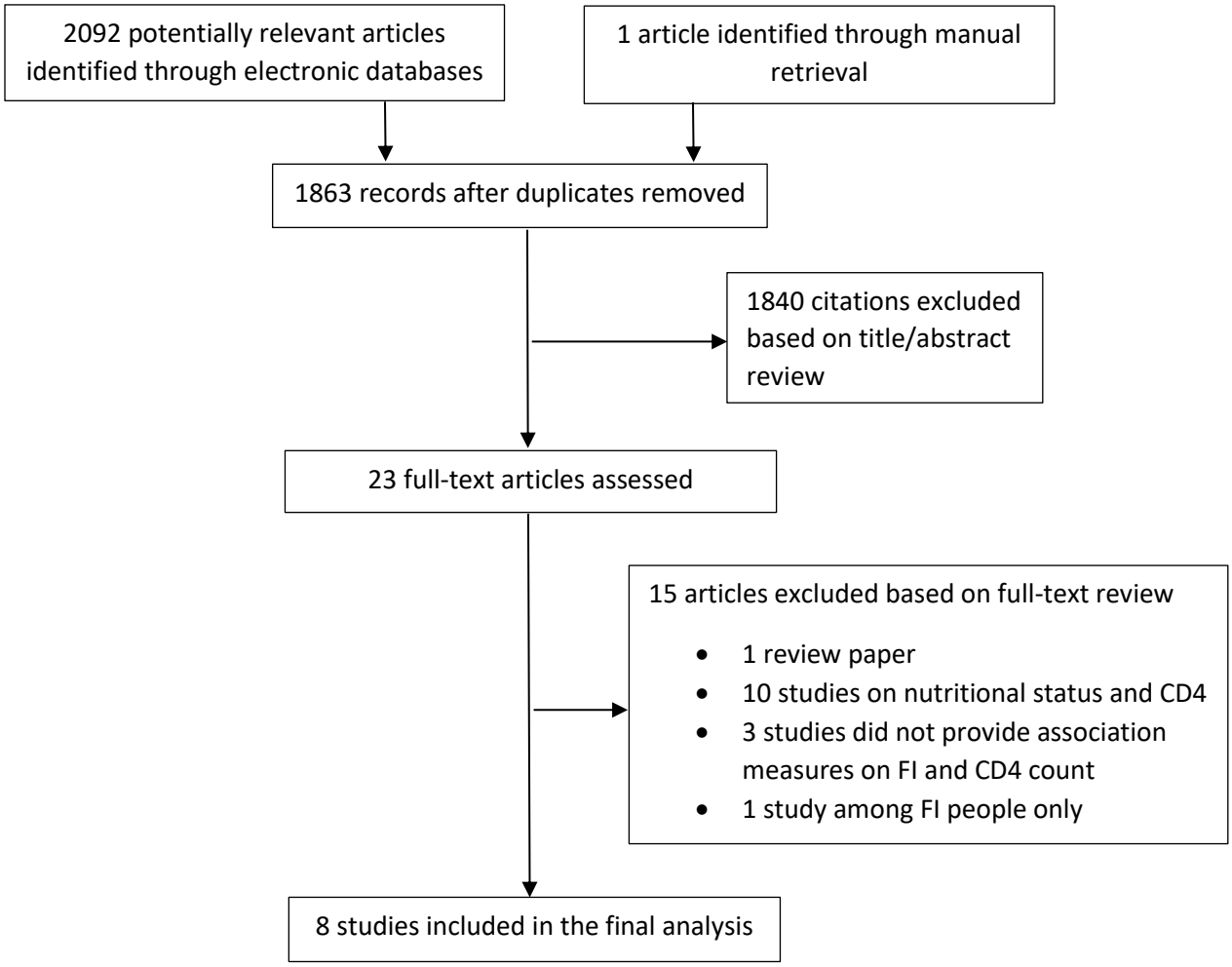


Figure 2.2.2 Pooled OR for food insecurity by study design

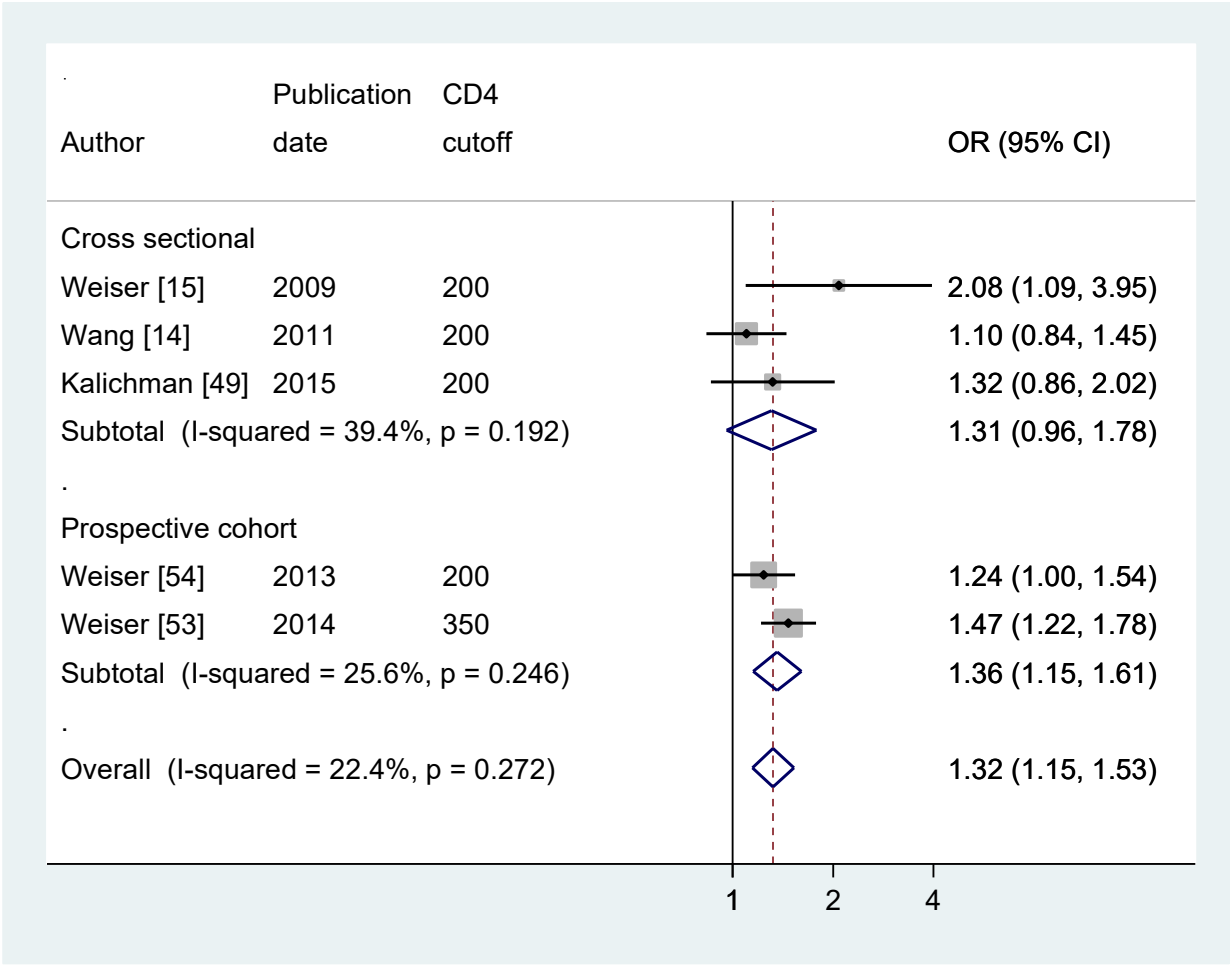
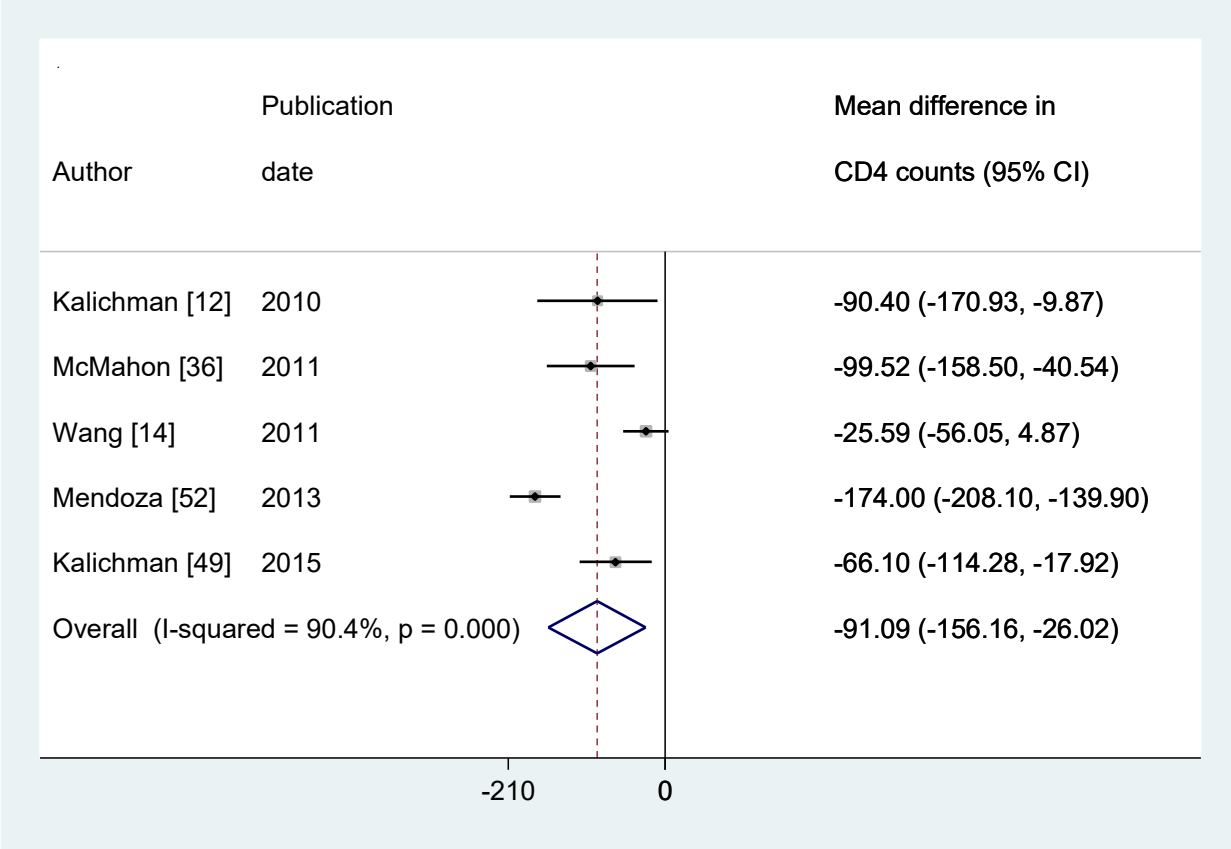


Figure 2.2.3 Pooled mean difference of CD4 count by exposure types



Appendix 2.2.1 Literature Search Strategy

PubMed

1. ((((((((((((((((((human immunodeficiency viruses[MeSH Terms]) OR hiv[Text Word]) OR aids[Text Word]) OR human immunodeficiency virus[Text Word]) OR human immuno deficiency virus[Text Word]) OR human immuno-deficiency virus[Text Word]) OR human immunodeficiency viruses[Text Word]) OR human immuno deficiency viruses[Text Word]) OR human immuno-deficiency viruses[Text Word]) OR acquired immunodeficiency syndrome[Text Word]) OR acquired immuno deficiency syndrome[Text Word]) OR acquired immuno-deficiency syndrome[Text Word]) OR acquired immunodeficiency syndromes[Text Word]) OR acquired immuno deficiency syndromes[Text Word]) OR acquired immuno-deficiency syndromes[Text Word])))))

2. ((((((food security[MeSH Terms]) OR hunger[MeSH Terms]) OR malnutrition[MeSH Terms]) OR food security[Text Word]) OR hunger[Text Word]) OR malnutrition[Text Word])

3. (((((((cd4 cell count[MeSH Terms]) OR cd4 cell counts[MeSH Terms]) OR cd4 count[MeSH Terms]) OR cd4 counts[MeSH Terms]) OR cd4 cell count[Text Word]) OR cd4 cell counts[Text Word]) OR cd4 count[Text Word]) OR cd4 counts[Text Word])

4. #1 AND #2 AND #3

Yielded 215 citations as of May 15th 2015.

Web of Science

1. TS=(hiv OR human immunodeficiency virus* OR human immuno deficiency virus* OR human immuno-deficiency virus* OR aids OR acquired immunodeficiency syndrome* OR acquired immuno deficiency syndrome* OR acquired immuno-deficiency syndrome*)

2. TS=(food security OR food securities OR food insecurity OR food insecurities OR nutrition OR hunger)

3. TS=(cd4 or cd4 count* OR cd4 cell count*)

4. #1 AND #2 AND #3

Yielded 231 citations as of May 15th 2015.

ProQuest ABI/INFORM Complete (1971-current)

(hiv OR human immunodeficiency virus* OR human immuno deficiency virus* OR human immunodeficiency virus* OR aids OR acquired immunodeficiency syndrome* OR acquired immuno deficiency syndrome* OR acquired immunodeficiency syndrome*) AND (food security

OR food securities OR food insecurity OR food insecurities OR nutrition OR hunger) AND (cd4 OR cd4 count* OR cd4 cell count*)

Yielded 135 citations as of May 15th 2015.

Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily, Ovid MEDLINE(R) and Ovid OLDMEDLINE(R) 1946 to Present

1. HIV Antigens/ or HIV Infections/ or HIV-1/ or HIV Seropositivity/ or HIV/ or HIV Antibodies/

2. Acquired Immunodeficiency Syndrome/bl, cf, di, me, mo, nu [Blood, Cerebrospinal Fluid, Diagnosis, Metabolism, Mortality, Nursing]

3. #1 OR #2

4. food insecurity.mp.

5. CD4 Lymphocyte Count/

6. #3 AND #4 AND #5

Yielded 16 citations as of May 15th 2015.

Embase classic+Embase

1. hiv.mp. or Human immunodeficiency virus/

2. aids.mp. or acquired immune deficiency syndrome/

3. #1 OR #2

4. food security.mp. or food security/ or food/ or nutrition/

5. malnutrition/ or health/ or hunger/ or food insecurity/ or starvation/ or socioeconomics/ or nutritional status/ or food intake/

6. #4 OR #5

7. cd4.mp. or CD4 antigen/

8. cd4 count.mp. or CD4 lymphocyte count/

9. #7 OR #8

10. #3 AND #6 AND #9

Yielded 1495 citations as of May 15th 2015.

Appendix 2.2.2 Adapted version of Newcastle Ottawa Scale (NOS) for assessing cross sectional study quality (Adaptation was based on work by Herzog, et al [70] with slight modification).

Selection: (Maximum 4 stars)

1) Representativeness of the sample:
   a) Truly representative of the average in the target population. * (all subjects or random sampling)
   b) Somewhat representative of the average in the target population. * (non-random sampling)
   c) Selected group of users.
   d) No description of the sampling strategy.

2) Sample size:
   a) Justified and satisfactory. *
   b) Not justified.

3) Non-respondents:
   a) Comparability between respondents and non-respondents characteristics is established, and the response rate is satisfactory. *
   b) The comparability between respondents and non-respondents is unsatisfactory.
   c) No description of the response rate or the characteristics of the responders and the non-responders.
   d) No description of respondents and non-respondents regarding important confounders.

4) Ascertainment of the exposure (risk factor):
   a) Validated measurement tool or the tool is available or described. *
   b) No description of the measurement tool.

Comparability: (Maximum 2 stars)

1) Comparability of different exposure groups except that exposure variable on the basis of the design or analysis:
   a) The study controls for the most important factor through regression or matching. *
   b) The study control for any additional factor. *

Outcome: (Maximum 3 stars)

1) Assessment of outcome.
   a) Independent blind assessment. **
   b) Record linkage. **
   c) Self-report. *
   d) No description.

2) Statistical test:

a) The statistical test used to analyze the data is clearly described and appropriate, and the measurement of the association is presented, including confidence intervals and the probability level (p value). *

b) The statistical test is not appropriate, not described or incomplete.

Appendix 2.2.3 Standard Error (SE) Formula used for Mean Differences Calculation

SE=$\sqrt{\frac{{S_1}^2}{n_1}+\frac{{S_2}^2}{n_2}}$,

where ${S_1}^2$ and ${S_2}^2$ are variances of CD4 counts in each of the groups, and n_1 and n_2 are the sample sizes of each group

2.3 Manuscript 2

Association between Food Insecurity and HIV Viral Suppression: A Systematic Review and Meta-Analysis

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Publication status: AIDS and Behavior. 2017 Mar;21(3):754-765.

Abstract

Although an increasing number of HIV infected people are accessing combined antiretroviral treatment (cART), many do not achieve complete HIV viral suppression and remain at risk for Acquired immune deficiency syndrome (AIDS) and capable of HIV transmission. Food insecurity has been identified as a potential risk factor for poor virologic response, but the associations between them have been inconsistent. We systematically searched five electronic databases and bibliographies of relevant studies through April 2015 and retrieved studies that quantitatively assessed the association between food insecurity and HIV viral load suppression. Meta-analyzed results revealed that experiencing food insecurity resulted in 29% lower odds of achieving complete HIV viral suppression (OR=0.71, 95% CI 0.61-0.82). This significant inverse association was consistently found regardless of study design, exposure measurement, and confounder adjustment methods. However, in order to have policy implications, future studies are needed to incorporate temporal relationship and using causal methodologies to account for time varying confounding.

Keywords: food insecurity, HIV infection, viral suppression, meta-analysis.

Introduction

HIV infection continues to be a major global public health issue. It was estimated in 2015 that nearly 37 million people globally were living with HIV [16]. Due to developments in HIV treatment programs in recent years, nearly half of HIV infected people are accessing combined antiretroviral treatment (cART) [16]. Since there is no cure for HIV infection, complete HIV viral suppression is a measure of treatment success and the primary goal of cART. However, recent studies have found that only about 75% of those who were receiving cART achieved complete HIV viral suppression [48-50], and this percentage declined with longer treatment duration [48]. Since HIV viral load is strongly associated with both vertical and horizontal transmission of HIV [88,89], those who have incomplete HIV viral suppression remain capable of forward transmission.

Poor treatment adherence and drug resistance are known risk factors for incomplete HIV viral suppression and need to be addressed accordingly. Recently, food insecurity has been identified as a potential risk factor for poor virologic responses among people living with HIV [28,31]. Food insecurity is defined as "limited or uncertain access to nutritionally adequate and safe foods or limited or uncertain availability to acquire such foods in socially acceptable ways" [1] and is measured with validated scales. The relevant time frame may vary from four weeks [28,78,90] to one year [2,29] depending on the actual scales employed. Although statistics about the prevalence of food insecurity among HIV infected people is limited, several locally conducted studies in North America showed that the prevalence of FI among HIV infected people was high and increasing over time [24,32].

Previous studies have demonstrated that people experiencing food insecurity were less likely to adhere their prescribed medications [56,57,75,90], probably due to disruption of daily routines, impairment of memory and attention, and reduction of motivation [91,92]. In addition to the behavioral pathway, plausible nutritional pathway has also been proposed. For example, when people experience food insecurity, different coping behaviors, such as reducing one's quality and quantity of food consumption [58,59,93] may make it difficult to meet minimum nutritional requirements, leading to nutritional deficiencies over the long term [60]. Micronutrient deficiency may compromise host immunity against HIV virus, leading to rapid virus replication and clinical progression of disease [61,62].

Despite the plausible mechanisms that may link food insecurity with poor virologic responses, the results are inconsistent across studies [28,31,75,76,94]. For example, while some studies have demonstrated that food insecurity is significantly associated with incomplete HIV viral suppression [28,31], others have failed to identify a significant association [75,76,94]. Potential reasons for this discrepancy could be attributed to differences in: study designs and study populations, sample sizes, the types of confounding variables adjusted for in each study, and the instruments used to measure food insecurity.

The identification of risk factors for incomplete HIV viral suppression is necessary in achieving ideal treatment outcomes and reducing HIV transmission. In order to summarize the existing evidence describing the association between food insecurity and complete HIV viral suppression, we conducted a systematic review and meta-analysis of epidemiologic studies with the aim of overcoming the inconsistencies in previous evaluations of this relationship. The primary research question of this review is: "What is the association between food insecurity

and complete HIV viral suppression?" In addition, we also assessed the robustness of this association across subgroups.

Methods

Search Strategy and Selection Criteria

The review was conducted in accordance with the PRISMA guidelines for reporting [95]. We have developed study protocol with clear statement of questions being addressed with reference to Participants, Interventions, Comparisons, and Outcomes (PICO). Based on the protocol, we have systematically searched electronic databases (PubMed, Web of Science, ProQuest ABI/INFORM Complete, Ovid MEDLINE(R) and EMBASE Classic) from the date of database inception to April 15th, 2015, to identify quantitative epidemiologic studies that assessed the association between food insecurity and HIV viral suppression (the full search strategy is provided in Appendix 2.3.1). Two authors (W.A. and T.M.) independently retrieved potentially relevant articles with full text based on title and abstracts screening and conducted article reassessment for eligibility through full text review. Studies were included according to the following criteria: (1) the study examined the association between food insecurity and complete HIV viral suppression among HIV infected people regardless of their cART status (2) the study provided raw data to calculate an unadjusted odds ratio (OR) if the adjusted association was not provided, (3) the study was peer-reviewed and written in English, French or Chinese, and (4) the study was conducted among an adult population. Studies were not excluded on the basis of study design or population, sample size, exposure or outcome

measurement method, or geographical region. The study selection process is detailed in Figure 2.3.1.

Data Extraction and Quality Assessment

A standardized data extraction form was developed. Two reviewers (W.A. and T.M.) independently extracted information on first author, publication year, study design, geographic region, sample size, number of exposed and unexposed participants, measures of association (i.e., adjusted ORs), and other adjustment variables (such as age, sex, race, income, education, marital status, employment, homelessness, drug use, smoking, alcohol, nadir CD4 count, incarceration, depression, current regimen type, prior HAART before study entry, mono/dual NRTI exposure before HAART, adherence). The Newcastle Ottawa Scale (NOS) [69] was used to assess study quality for cohort studies and an adapted version was used for cross-sectional studies [70] (Appendix 2.3.2 is adapted version of NOS for cross-sectional studies; Appendix 2.3.3 is scoring details for each included studies). The NOS is composed of three domains: sample selection, comparability, and outcome measurement; each domain contains several aspects that collectively support the quality of the domain. For example, in comparability, adjusting for confounders is an important aspect, leading to a higher score for that domain. The overall score ranges between 0 and 9 stars; more stars representing better quality. Upon completion of data extraction, study qualities were compared and discrepancies were resolved through consensus.

Study Outcomes

Measures of association (expressed as ORs) were directly used if the original article presented the association between food insecurity and *complete* HIV viral suppression (HIV viral suppression is often defined by a certain threshold, e.g., less than 50 copies/mL, and this threshold may differ across studies). In studies presenting the association between food insecurity and *incomplete* HIV viral suppression, the reported ORs were inverted. For example, if a study reported the association between food insecurity and *incomplete* HIV viral suppression as OR=1.24 (95% CI 0.99-1.55) [28], it was inverted as OR=0.81 (95% CI 0.65-1.01) to represent the association between food insecurity and *complete* HIV viral suppression. Additionally, attempts were made to obtain an adjusted OR by contacting study authors. When author contacts were unsuccessful, unadjusted ORs and 95% confidence intervals (CIs) were calculated using two by two tables. Standard errors were used to construct forest plot (Figure 2.3.2).

Statistical Analysis

In order to allow for underlying differences in study design, study participants and study settings, pooled ORs were estimated using a DerSimonian and Laird random effects model, where study weight is inversely proportional to study variance [73]. Heterogeneity between studies was assessed using the Higgins' I^2 test [72], a quantity ranging from 0-100%. A larger I^2 indicates that the total variation between studies is due to true heterogeneity rather than chance [72]. Subgroup analyses were conducted to examine robustness of the pooled association, where subgroups were defined based on confounder adjustment methods, study design, food insecurity measurement methods, study quality based on NOS assessment, and viral suppression threshold. To identify publication bias, funnel plot and meta-bias analyses

were conducted. The Egger test method [96] for both meta-bias analysis and funnel plot construction was used. Lastly, sensitivity analyses were conducted by omitting one study at a time to explore whether the pooled estimates were strongly influenced by a single study. All analyses were completed using Stata (Stata Corporation, Version 12.0, College Station, TX, USA).

Results

The electronic database search identified 2134 citations and one citation was identified through manual reference search. Of the 2064 de-duplicated records, 2040 were excluded after title/abstract screening, resulting in 24 records for full text screening. The final analysis included 11 studies that met the inclusion criteria (Figure 2.3.1).

Table 2.3.1 describes the main characteristics of each eligible study. Studies were published between 2009 and 2015 in the United States (7 studies), Canada (2 studies), Uganda (1 study), and Brazil (1 study). Seven studies were cross sectional [26,28,29,31,90,94,97] and four were cohort studies [75,76,98,99]. The total sample size of the 11 included studies was 7562, where individual studies' sample sizes ranged from 103 [97] to 2353 [31] with a median of 406 participants. Mean (or median) ages varied between 35 and 51 years. Seven studies [28,31,75,76,90,94,98] reported adjusted ORs for complete HIV viral suppression. After author contacts for adjusted ORs were unsuccessful, ORs for the remaining four studies [26,29,97,99] were calculated using two by two tables.

Study quality varied widely across studies with NOS ratings ("stars") ranging from 1 to 8 stars. In general, studies that did not provide adjusted ORs for complete viral suppression tended to be of lower quality (Table 2.3.1). Information regarding losses to follow-up was

unavailable for two [75,98] of the four [75,76,98,99] cohort studies, whereas the remaining two studies reported that <20% of their participants were lost [76,99]. None of the seven cross sectional studies [26,29,31,32,90,94,97] provided a comparison between respondents and non-respondents with regards to important characteristics, such as age distribution, level of education, or employment status.

With the exception of one study [99], all included studies measured food insecurity status using a validated scale. However, only four studies used the originally validated scale in its entirety [28,75,76,94], whereas the remaining studies used a portion of the scale [26,29,31,90,97,98] (Table 2.3.1). In the latter case, the validity of the scale is unclear. The relevant time frame varied from four weeks [28,75,90] to one year [29]. Five studies [28,31,75,76,90] used the Household Food Insecurity Access Scale (HFIAS) [78], and among them, one study used only the first item of the scale [31]; another study used four items from the original scale [90]. Four studies [26,29,97,98] used an adapted version of the US Department of Agriculture's Household Food Security Survey Module (HFSSM) [2]. One study [94] used the Radimer/Cornell food insecurity scale [77]. Regarding the outcome variable, two studies [29,97] used patient-reported HIV viral load while the remaining studies used laboratory reported results.

The pooled association between food insecurity and complete HIV viral suppression is presented in Figure 2.3.2. Despite individual study results varying widely from OR=0.23 (95% CI 0.06-0.85) to OR=1.04 (95% CI 0.74-1.47), the overall pooled estimate indicates that people experiencing food insecurity had 29% lower odds of achieving complete HIV viral suppression

than people experiencing food security (OR=0.71, 95% CI 0.61-0.82, Z=4.60, P=0.000). The I^2 value was 26.6% (X^2=13.63, df=10, P=0.19), indicating low heterogeneity between studies.

With the exception of the subgroup that measured food insecurity using a complete measurement scale, significant inverse associations between food insecurity and complete HIV viral suppression were observed in all subgroups (Table 2.3.2). Studies that adjusted for potential confounders tended to report weaker associations than those that did not adjust for confounders. Cohort studies reported weaker associations than cross-sectional studies. Studies that measured food insecurity with a complete measurement scale reported marginally non-significant associations, while studies using a portion of an originally validated scale reported significant inverse associations. However, we did not detect significant heterogeneity in any of the subgroups (i.e., all three values of $P_{\text{heterogeneity}}$[b] > 0.05 in Table 2.3.2).

In sensitivity analyses, the exclusion of any one study from the analyses did not substantially alter the pooled estimate and the estimate remained statistically significant (Figure 2.3.3). The pooled estimate for complete HIV viral suppression ranged from OR=0.68 (95% CI 0.57-0.81; when the study by Weiser SD, et al [76] was excluded) to OR=0.73 (95% CI 0.63-0.85; when the study by Kalichman SC, et al [26] was excluded). The results were similar when using only adjusted estimates (results was not shown).

We examined publication bias by plotting the log transformed association measures (ORs) against their standard errors. In Figure 2.3.4, there is a strong suggestion of asymmetry in the funnel plot, suggesting that small studies with null and positive findings (small studies that either did not find or found positive association between food insecurity and complete viral

suppression) are missing on the right hand side. The Egger's linear regression test also detected significant publication bias (t=-2.96, P=0.016). We used the trim-and-fill method [100] to correct for publication bias and it resulted in no studies needing to be filled.

Discussion

Identifying and quantifying the effect of risk factors for incomplete HIV viral suppression is important in order to identify patients who are potentially at higher risk for virologic failure and to reduce HIV transmission. The combined evidence from this meta-analysis indicated that food insecurity is inversely associated with complete HIV viral suppression. Comparing people who experienced food insecurity with those who did not, the odds of having complete HIV viral suppression decreased by 29% (OR=0.71, 95% CI 0.61-0.82, Z=4.60, P=0.000). The direction of this association was markedly consistent across all subgroups (Table 2.3.2) and it did not change in the sensitivity analyses (Figure 2.3.3), adding further support to our main conclusions.

Although both behavioral [56,57,75,92] and nutritional studies [59-62,93] support the plausibility of these findings, it may still be difficult to claim causal association between food insecurity and complete HIV viral suppression. Temporality is a fundamental requirement for establishing causal relationship [101]. This meta-analysis included seven cross-sectional studies and four cohort studies. It is impossible to have clear temporal order between exposure and outcome in cross-sectional study design. Cohort study design, on the other hand, assures temporality between exposure and outcome. However, in the repeated measurement settings, it is possible to have time-varying confounders affected by prior exposure. In this setting, the standard adjustment method, including GEE may be biased [102] and using methods designed

for this situation, such as the Marginal Structural Model (MSM) or other causal methods [86,103] may be more appropriate. However, none of the included cohort studies have clear statement on how potential time varying confounders were addressed.

In subgroup analyses, studies that measured food insecurity using the complete validated scale (as opposed to only using a portion of the scale) reported non-significant associations between food insecurity and complete HIV viral suppression (Table 2.3.2). This was unexpected as we would predict that such an attenuation of effect might be more likely to occur in studies that used only a portion of the validated scale. This is due to the fact that non-differential misclassification of the exposure (potentially due to altered psychometric properties of the partial scale) may cause an underestimation of the association [104]. However, among the subgroup of studies using the complete scale, all four studies [28,75,76,94] adjusted for medication adherence, and three of them [75,76,94] reported non-significant associations between food insecurity and complete HIV viral suppression. A prior cohort analysis showed that food insecurity is associated with poor medication adherence among people living with HIV [75] and poor medication adherence is direct cause for HIV viral non-suppression. Therefore, medication adherence is likely an intermediate variable in the pathway between food insecurity and HIV viral suppression, adjusting for it could lead to over-adjustment and bias the association towards the null [105]. However, it is important to keep in mind that poor medication adherence may also cause food insecurity perhaps through worsened health status, and so act as a confounder for the association between food insecurity and incomplete viral suppression. Therefore, it is appropriate to adjust for medication adherence in a cohort study with point exposure and point outcome measurement if medication adherence is measured at

baseline. In a cohort study with repeated measurement, however, it is better to use lagged exposure to assure correct time order between food insecurity and medication adherence and only adjust for medication adherence that is measured prior to the current exposure measurement. However, regression adjustment may not be appropriate in this setting since medication adherence is likely a time varying confounder (medication adherence is likely affected by prior food insecurity status, while it affects the next food insecurity status, perhaps through worsened health status, and HIV viral suppression). Therefore, it may require causal inference methods [86,103].

There was some indication of publication bias both in the funnel plot (Figure 2.3.4) and in the meta bias analysis (t=-2.96, P=0.016). From figure 2.3.4 it can be supposed that an unknown number of small studies with positive association between food insecurity and complete viral suppression have failed to be published. Therefore, our pooled estimate is likely an over estimation of the actual association. However, the Trim-and-fill method [100], used to identify theoretically missing studies in the funnel plot, resulted in no studies needing to be filled, indicating that the impact of publication bias was minor. Nevertheless, including only published literature implies we have missed many unpublished literature and inclusion of grey literature would have corrected this publication bias. Therefore, it remains necessary to take this into account when interpreting the pooled estimate.

Strengths of this systematic review and meta-analysis include a broad search strategy involving five different electronic databases. Consequently, a large combined sample size (7562 participants) permitted several subgroup analyses to further explore this inverse association between food insecurity and complete HIV viral suppression. Lastly, included studies were

relatively recent, providing timely data on the association between food insecurity and HIV viral suppression. However, there are also several limitations. First, we could not rule out biases possibly caused by different food insecurity measurement scales. Different scales are expected to have different sensitivities and specificities in classifying food insecurity. However, exposure misclassification was likely non-differential due to the fact that the outcome was a laboratory measure. Non-differential misclassification of a dichotomous exposure tends to bias towards the null, likely indicating that our findings are conservative. Second, we only included data from published peer-reviewed sources and this could further contribute to publication bias. Third, although the included studies controlled for important confounders such as education, sex, and employment status, it is still possible to have residual confounding caused by unknown confounders and/or imprecise adjustment strategies. Fourth, it is also important to notice the fact that more than half of the included studies came from two research groups, meaning that included studies resemble each other more than independent studies conducted by different research groups, and resulted in overly narrow confidence intervals around pooled estimate. Lastly, due to the fact that the majority of the included studies were based in North America, the pooled estimate may have limited generalizability to resource poor settings.

Incomplete HIV viral suppression poses a substantial threat to both personal health status and HIV control efforts through deterioration of the hosts' immune system and the potential for ongoing HIV transmission in the community. This systematic review and meta-analysis of epidemiologic studies indicated that food insecurity is inversely associated with complete viral suppression. However, it is difficult to claim causal association due to the fact that temporality was not clear among the cross sectional studies and adjustment method for

potential time varying confounders was not clearly stated among cohort studies. Therefore, in order to have policy implication, future studies are needed to incorporate temporal order between exposure and outcome, and using more robust methodologies to account for time varying confounding.

Table 2.3.1 Characteristics of the 11 included studies describing the association between food insecurity and HIV viral suppression published between 2009 and 2015

Author	Year	Study design	Country	Sample size	Age (SD or IQR)	Exposure definition	Exposure measurement	Adjustment variables	Quality [69]
Weiser SD [28]	2009	Cross sectional	USA	104	46.5 (7.9)	Severe food insecurity	9 item HFIAS	age, sex ,race, income, education employment, recent homelessness, drug use over the past 30 days, nadir CD4 count, problem drinking, incarceration, depression, current regimen type, prior HAART before study entry, mono/dual NRTI exposure before HAART, adherence	7 stars
Wang EA [31]	2011	Cross sectional	USA	2353	49.5 (8.5)†	Any food insecurity	First question of 18 item HFIAS	age, gender, race/ethnicity, income, education, marital status, employment status, homelessness, AUDIT-C alcohol use, drug use, cART adherence	7 Stars

Weiser SD [76]	2013	Cohort	USA	284	48 (IQR 43-54)	Any food insecurity	9-item HFIAS	1. Time fixed: Sex, age, ethnicity, income, education, months on cART at baseline, nadir CD4 count, cART adherence, VL level. 2. Time varying: recent homelessness, health insurance status, illicit drug use, alcohol, smoking	8 Stars
Anema A [94]	2014	Cross sectional	Canada	406	44.4 (IQR 38.9-48.8)	Severe food insecurity	Radimer/ Cornell	age, gender, aboriginal ancestry, homelessness, education, income, drug use, alcohol use, depression, year of cART, baseline HIV RNA, adherence	8 Stars
Weiser SD [75]	2014	Cohort	Uganda	438	35.4 (8.5)	Any food insecurity	9-item HFIAS	1. Time fixed: Age, sex, marital status, education, cART status at baseline, Pre-ART CD4. 2. Time varying: cART adherence, Viral suppression, employment, household asset index, alcohol drinking and smoking	8 Stars
Kalichman SC [29]	2010	Cross sectional	USA	344	44.4 (7.8)†	Any food insecurity	6 of 18 items HFSSM	None	3 Stars
Charão AP [97]	2012	Cross sectional	Brazil	103	42.6 (10.1)	Any food insecurity	6 of 18 items HFSSM	None	1 Stars

Kalichman SC [90]	2014	Cross sectional	USA	183	45.9 (7.4)†	Any FI	4 items out of HFIAS	age, gender, education, employment, years HIV+ drug use, alcohol use, depression,	6 Stars
Kalichman SC [26]	2015	Cross sectional	USA	759	48 (8.5)†	Any FI	8 items adapted from HFSSM	None	4 Stars
Shannon K [99]	2011	Cohort	Canada	470	42 (IQR 36-47)	Severe FI	NS	None	6 Stars
Feldman MB [98]	2015	Cohort	USA	2118	51.1 (10.2) †	Any FI	3 items adapted from HFSSM	age, gender, education, employment, housing situation, years HIV+ drug use, BMI	8 Stars

Notes and Abbreviations: The Radimer/Cornell Food Insecurity scale was developed by Radimer, et al (1992) and modified and validated by Kendall, et al (1995) (Household level and Individual level; the above study used the Individual/adult level part); HFIAS, Household Food Insecurity Access Scale (Household level); HFSSM, Household Food Security Survey Module (Household level); NRTI, Nucleoside Reverse Transcripts Inhibiter; † indicates that age and standard deviation were provided separately by exposure status in original papers, these values were combined using sample mean and standard deviation combining formulas; NS, Not stated.

Table 2.3.2 Odds ratios describing the association between food insecurity and complete HIV viral suppression in the 11 included studies published between 2009 and 2015, categorized by subgroups

Subgroups		Number of studies	OR	95% CI	$P_{heterogeneity}$[a]	$P_{heterogeneity}$[b]
All studies		11	0.71	0.61, 0.82	0.19	
Adjusted	Yes	7	0.74	0.61, 0.90	0.114	0.179
	No	4	0.62	0.49, 0.78	0.859	
Study design	Cohort	4	0.8	0.66, 0.97	0.246	0.144
	Cross sectional	7	0.64	0.53, 0.78	0.356	
FS measured with full scale	Yes	4	0.78	0.57, 1.07	0.099	0.133
	No	7	0.67	0.57, 0.78	0.654	
Study quality	7 or more stars	6	0.77	0.64, 0.92	0.17	0.091
	6 or less stars	5	0.6	0.48, 0.75	0.735	
Viral suppression threshold	<200	5	0.65	0.51, 0.83	0.174	0.382
	<500	5	0.77	0.61, 0.97	0.216	

Notes: a, P value for heterogeneity within each subgroup; b, P value for heterogeneity between subgroups in meta-regression analysis; †, among this subgroup, one study did not state how food insecurity was measured, the remaining studies used a portion of originally validated measurement scale rather than using the complete scale.

Figure 2.3.1 Flow chart of study selection process

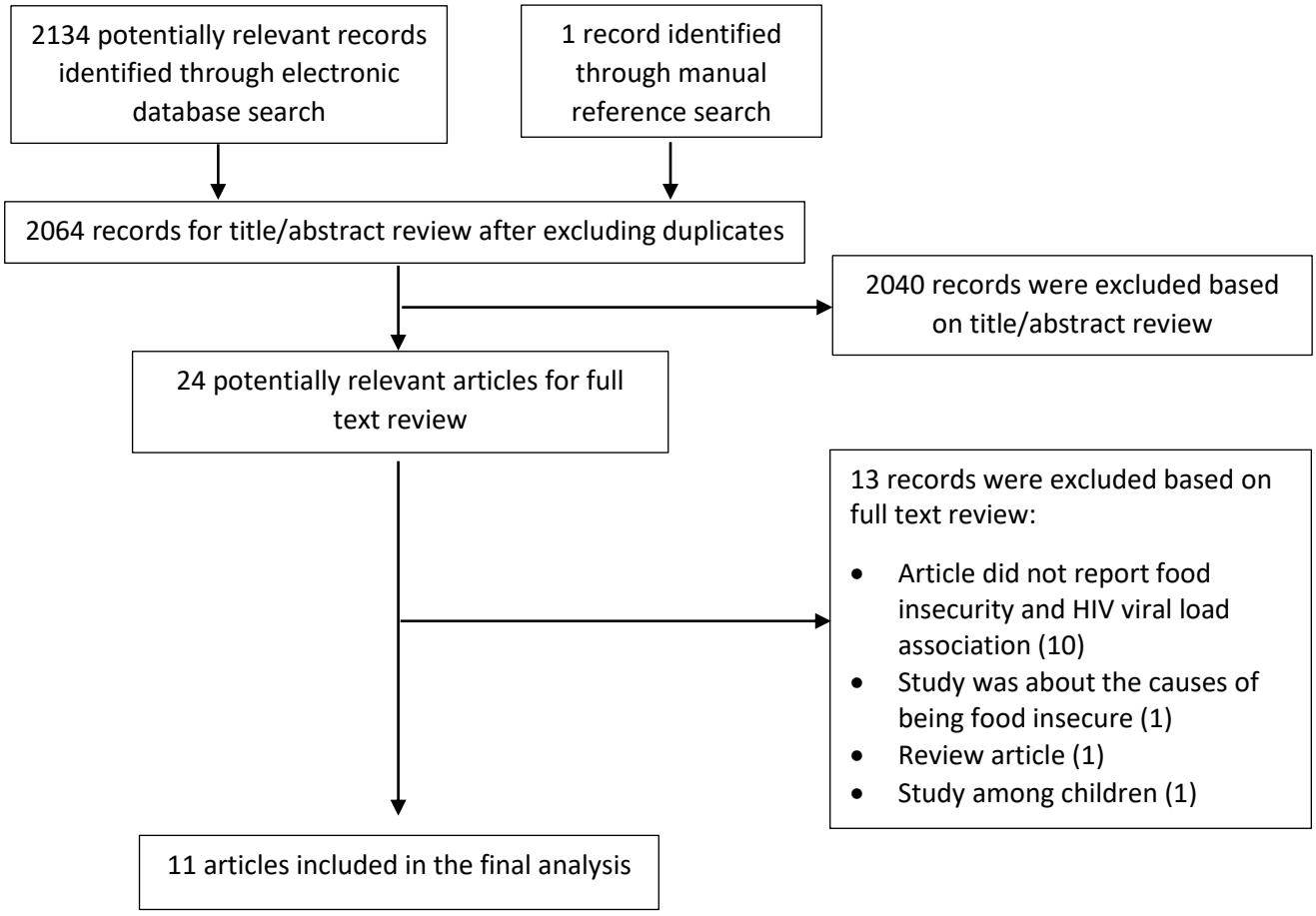
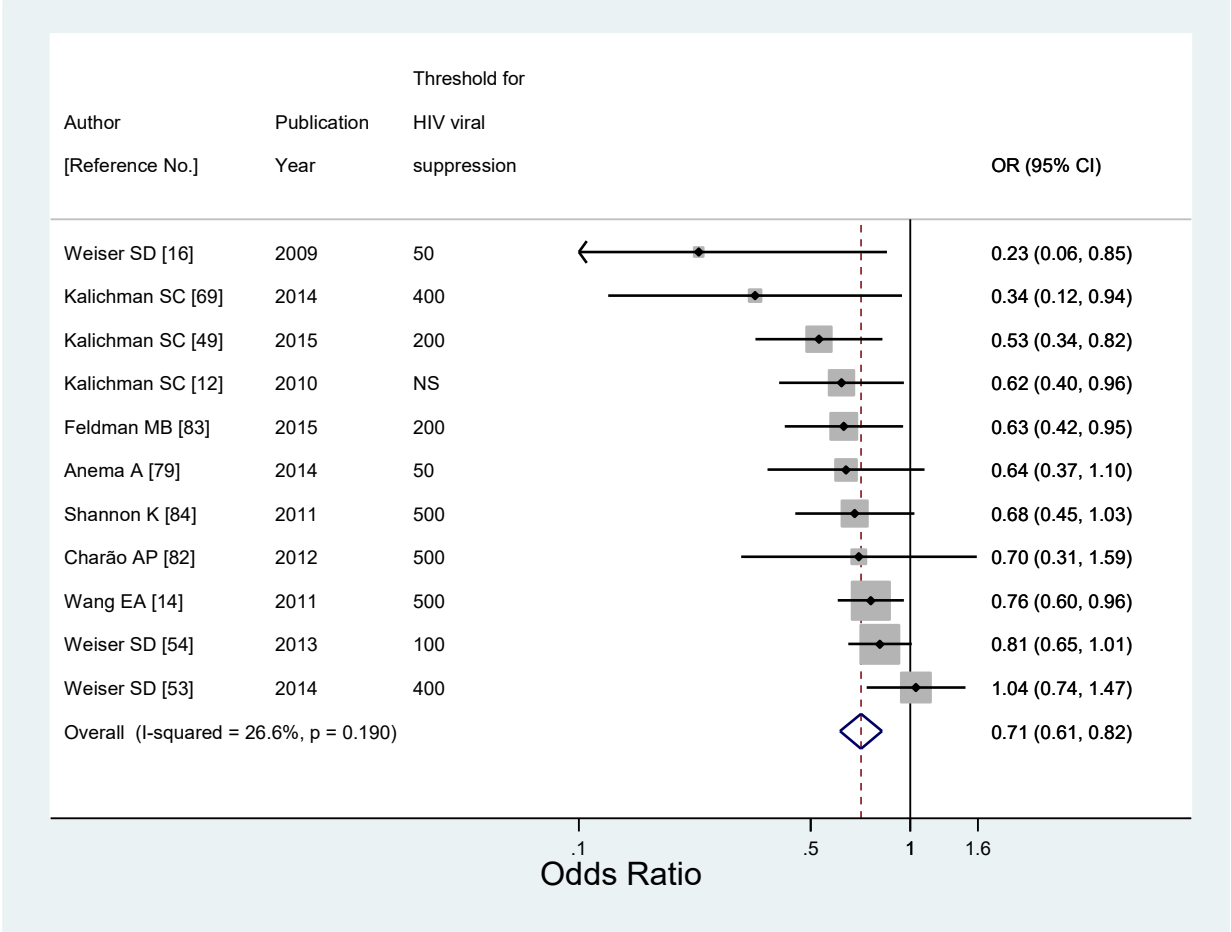


Figure 2.3.2 Pooled odds ratio for complete HIV viral suppression across 11 studies describing the association between food insecurity and HIV viral suppression published between 2009 and 2015



Notes and Abbreviations: NS, Not stated.

Figure 2.3.3 Sensitivity analyses for assessing the impact of individual studies on the pooled estimate

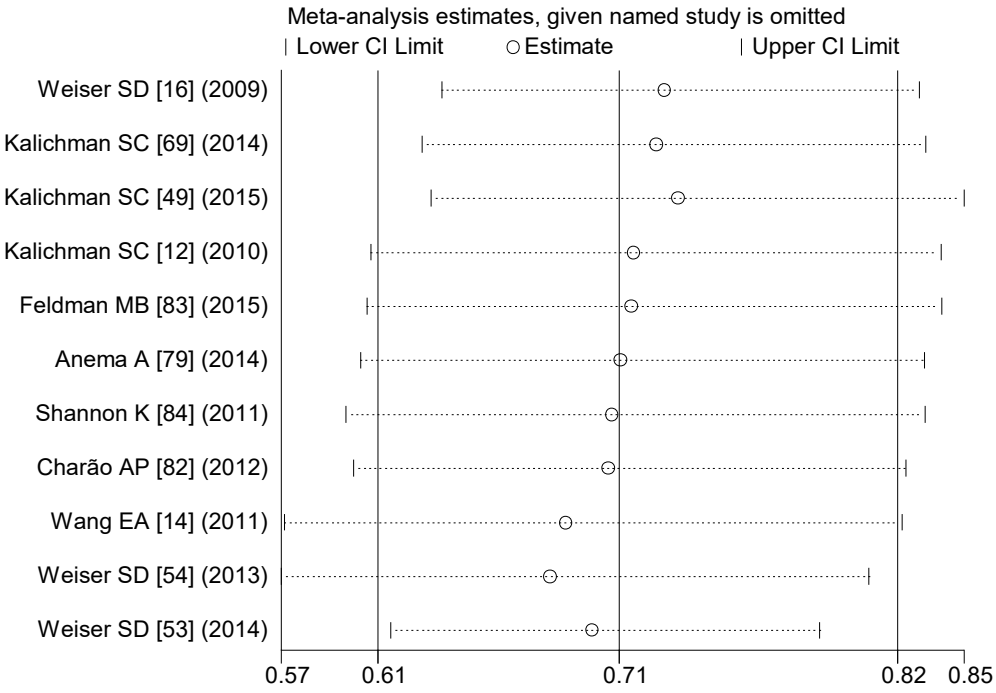
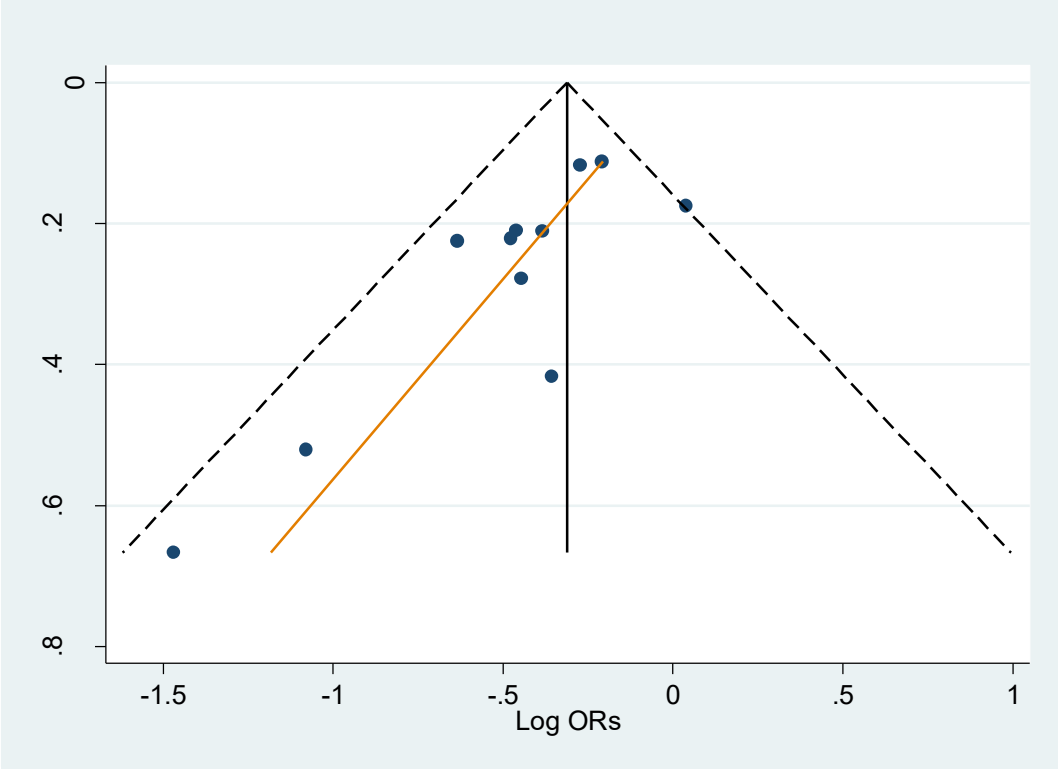


Figure 2.3.4 Funnel plot of the 11 included studies describing the association between food insecurity and HIV viral suppression published between 2009 and 2015



The orange line in the funnel plot is a regression line corresponding to the regression test for funnel-plot asymmetry proposed by Egger et al[34].

Appendix 2.3.1 Literature Search Strategy

PubMed

1. (human immunodeficiency viruses[MeSH Terms] OR hiv[Title/Abstract] OR aids[Title/Abstract] OR human immunodeficiency virus[Title/Abstract] OR human immuno deficiency virus[Title/Abstract] OR human immuno-deficiency virus[Title/Abstract] OR human immunodeficiency viruses[Title/Abstract] OR human immuno deficiency viruses[Title/Abstract] OR human immuno-deficiency viruses[Title/Abstract] OR acquired immunodeficiency syndrome[Title/Abstract] OR acquired immuno deficiency syndrome[Title/Abstract] OR acquired immuno-deficiency syndrome[Title/Abstract] OR acquired immunodeficiency syndromes[Title/Abstract] OR acquired immuno deficiency syndromes[Title/Abstract] OR acquired immuno-deficiency syndromes[Title/Abstract])

2. (food security[MeSH Terms] OR hunger[MeSH Terms] OR malnutrition[MeSH Terms] OR food security[Title/Abstract] OR malnutrition[Title/Abstract] OR hunger[Title/Abstract] OR food insufficienc*[Title/Abstract])

3. (viral load[MeSH Terms] OR viral rna[MeSH Terms] OR viral load[Title/Abstract] OR viral rna[Title/Abstract] OR virus load[Title/Abstract] OR virus rna[Title/Abstract])

4. 1 AND 2 AND 3

101 citations as of April 15th, 2015

Web of Science

1. TS=(hiv OR human immunodeficiency virus* OR human immuno deficiency virus* OR human immuno-deficiency virus* OR aids OR acquired immunodeficiency syndrome* OR acquired immuno deficiency syndrome* OR acquired immuno-deficiency syndrome*)

2. TS=(food security* OR food insecurity* OR nutrition* OR hunger* OR food insufficienc*)

3. TS=(viral load* OR viral rna* OR virus load OR virus rna)

4. 1 AND 2 AND 3

223 citations as of April 15th, 2015

ProQuest ABI/INFORM Complete

(hiv OR human immunodeficiency virus* OR human immuno deficiency virus* OR human immunodeficiency virus* OR aids OR acquired immunodeficiency syndrome* OR acquired immuno deficiency syndrome* OR acquired immunodeficiency syndrome*) AND (food security* OR food insecurity* OR hunger* OR food insufficient*) AND (viral load* OR viral rna* OR virus rna*)

Source type: Conference Papers & Proceedings, Dissertations & Theses, Scholarly Journals

342 citations as of April 15th, 2015

Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily, Ovid MEDLINE(R) and Ovid OLDMEDLINE(R) 1946 to Present

1. HIV Antigen/ or HIV Infection/ or HIV Seropositivity/ or HIV/ or HIV Antibody/

2. Acquired Immunodeficiency Syndrome/bl, cf, di, me, mo, nu [Blood, Cerebrospinal Fluid, Diagnosis, Metabolism, Mortality, Nursing]

3. #1 OR #2

4. food insecurity.mp.

5. food insufficiency.mp.

6. exp Malnutrition/ or exp Hunger/ or exp Food Supply/ or exp Poverty/

7. #4 OR #5 OR #6

8. exp HIV/ or exp Viral Load/

9. exp RNA, Viral/an, bl, du, de, im, ip, tu [Analysis, Blood, Diagnostic Use, Drug Effects, Immunology, Isolation & Purification, Therapeutic Use]

10. #8 OR #9

11. #3 AND #7 AND #10

270 citations as of April 15th, 2015

Embase classic+Embase

1. hiv.mp. or exp Human immunodeficiency virus/

2. exp acquired immune deficiency syndrome/di, dm, dr, dt, ep, et, pc [Diagnosis, Disease Management, Drug Resistance, Drug Therapy, Epidemiology, Etiology, Prevention]

3. #1 OR #2

4. exp malnutrition/ or exp hunger/ or exp food insecurity/ or exp starvation/ or exp socioeconomics/ or exp nutritional status/ or exp food intake/ or food insufficiency.mp.

5. viral load.mp. or exp virus load/

6. viral rna.mp. or exp virus RNA/

7. #5 OR #6

8. #3 AND #4 AND #7

1198 citations as of April 15th, 2015

Appendix 2.3.2 Adapted version of Newcastle Ottawa Scale (NOS) for assessing cross sectional study quality (Adaptation was based on work by Herzog, et al [22] with slight modification).

Selection: (Maximum 4 stars)

1) Representativeness of the sample:
   a) Truly representative of the average in the target population. * (all subjects or random sampling)
   b) Somewhat representative of the average in the target population. * (non-random sampling)
   c) Selected group of users.
   d) No description of the sampling strategy.

2) Sample size:
   a) Justified and satisfactory. *
   b) Not justified.

3) Non-respondents:
   a) Comparability between respondents and non-respondents characteristics is established, and the response rate is satisfactory. *
   b) The comparability between respondents and non-respondents is unsatisfactory.
   c) No description of the response rate or the characteristics of the responders and the non-responders.
   d) No description of respondents and non-respondents regarding important confounders.

4) Ascertainment of the exposure (risk factor):
   a) Validated measurement tool or the tool is available or described. *
   b) No description of the measurement tool.

Comparability: (Maximum 2 stars)

1) Comparability of different exposure groups except that exposure variable on the basis of the design or analysis:
   a) The study controls for the most important factor through regression or matching. *
   b) The study control for any additional factor. *

Outcome: (Maximum 3 stars)

1) Assessment of outcome.
   a) Independent blind assessment. **
   b) Record linkage. **
   c) Self-report. *
   d) No description.

2) Statistical test:

a) The statistical test used to analyze the data is clearly described and appropriate, and the measurement of the association is presented, including confidence intervals and the probability level (p value). *

b) The statistical test is not appropriate, not described or incomplete.

Appendix 2.3.3 Scoring details for each included studies

Cohort Studies

NOS items	Weiser SD [76]	Weiser SD [75]	Shannon K [99]	Feldman MB [98]
Representative of the exposed cohort	1	1	1	1
Selection of the non-exposed cohort	1	1	1	1
Ascertainment of exposure	1	1	0	1
Demonstration that outcome of interest was not present at start of study	1	1	1	1
Comparability of cohorts on the basis of the design or analysis	2	2	0	2
Assessment of outcome	1	1	1	1
Was follow-up long enough for outcomes to occur	1	1	1	1
Adequacy of follow up of cohorts	0	0	1	0

Cross Sectional Studies

NOS items	Weiser SD [28]	Wang EA [31]	Anema A [94]	Kalichman SC [29]	Charao AP [97]	Kalichman SC [90]	Kalichman SC [26]
Representativeness of the sample	1	1	1	1	0	0	1
Sample size justified	0	0	1	0	0	0	0
Non-respondents	0	0	0	0	0	0	0
Ascertainment of the exposure	1	1	1	1	1	1	1
Comparability of different exposure groups except that exposure variable on the basis of the design or analysis	2	2	2	1	0	2	0
Assessment of outcome	2	2	2	0	0	2	2
Statistical test	1	1	1	0	0	1	0

Chapter 3: Overall Association between Food Insecurity, and both HIV Viral Load and CD4 Count, among HIV-HCV Co-Infected People

3.1 Preface to Manuscript 3

In chapter 2, the results of the systematic review and meta analyses indicated that food insecurity (FI) is associated with poor virologic control and lower CD4 count among HIV infected people with unknown proportion of HCV co-infection. However, little is known about the HIV-related health outcomes of FI among HIV-hepatitis C virus (HCV) co-infected people. Although HIV mono-infected and HIV-HCV co-infected people share many characteristics, some other characteristics are unique to co-infected people. For example, they have higher rates of injection drug use (IDU) and liver related morbidity. These characteristics are also risk factors for uncontrolled HIV viral load and lower CD4 count. In the presence of additional risk factors, it is unclear if the same associations hold among co-infected people. In other words, it is difficult to generalize findings from HIV mono-infected people to HIV-HCV co-infected people because of the inherent differences between these population.

In addition, published research on the associations between FI and both HIV viral load and CD4 count have not explicitly accounted for potential time-varying confounders. In a cohort study involving repeated measures, both past HIV viral load and CD4 count can confound the subsequent associations between FI and both HIV viral load and CD4 count, and act as time-varying confounders. Inappropriate adjustment for time-varying confounders may introduce two layers of bias: blockage of at least partial exposure effects and introduction of a collider

stratification bias. The magnitude and direction of overall bias is determined by the magnitude and directions of these two layers of biases.

In manuscript 3, associations between FI and both HIV viral load and CD4 count among HIV-HCV co-infected people was assessed using inverse probability weighted marginal structural model. In addition to the main analysis, a series of sensitivity analyses were also conducted to assess robustness of the findings using different sets of confounders and different adjustment strategies.

This manuscript was presented at the department of Epidemiology, Biostatistics and Occupational Health 2017 Student Research Day in Montreal, Canada, in April 2017; at the Infectious Diseases and Immunity in Global Health (IDIGH) 2017 Trainee Research Day in Montreal, Canada, in April, 2017; and at the Society for Epidemiologic Research (SER) 50th Anniversary Meeting in Seattle, USA, in June 2017.

3.2 Manuscript 3

Food Insecurity May Lead to Incomplete HIV Viral Suppression and Less Immune Reconstitution among HIV-HCV Co-Infected People

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Publication status: HIV Medicine, *In press*

Abstract

***BCKGROUND*:** Food Insecurity (FI) is associated with unsuppressed HIV viral load and poor immune reconstitution among HIV infected people. It is unclear to what extent this association holds among people co-infected with HIV-hepatitis C virus (HCV).

***OBJECTIVE*:** To determine the impact of FI on HIV viral load and CD4 count among people co-infected with HIV-HCV.

***DESIGN AND PARTICIPANTS*:** A prospective cohort study conducted from 2012 to 2015. Participants were patients co-infected with HIV-HCV and receiving care at 18 treatment sites across 6 Canadian provinces.

MAIN MEASURE: Exposure variable was FI and measured using the adult scale of Health Canada's Household Food Security Survey Module and was classified into three categories: food secure, moderate food insecurity and severe food insecurity. Outcome variables were HIV viral load and CD4 count. HIV viral load was categorized as detectable vs. non-detectable (<50 copies/mL), CD4 count was natural log transformed continuous variable.

***KEY RESULTS*:** A total of 725 HIV-HCV co-infected people with 1973 person-visits over 3 years of follow up contributed to this study. At baseline, 23% of participants experienced moderate food insecurity and 34% experienced severe food insecurity. The proportion of people with undetectable HIV viral load was 75% and the median CD4 count was 460 (IQR: 300-665). People experiencing severe food insecurity had 1.47 times (95% CI: 1.14, 1.88) the risk of having detectable HIV viral load and 0.91-fold (95% CI: 0.84, 0.98) increase in CD4 count compared to people who were food secure.

***CONCLUSION*:** These findings provide evidence of the negative impact of food insecurity on HIV viral load and CD4 count among HIV-HCV co-infected persons.

KEY WORDS: HIV-HCV co-infection; food insecurity; HIV viral load; CD4 count; marginal structural model.

Introduction

Food insecurity (FI), defined as a limited or uncertain ability to acquire acceptable foods in socially acceptable ways, or limited or uncertain availability of nutritionally adequate and safe foods [106], is a significant social and health problem in Canada, where more than 8% of households experienced some level of FI in 2011-2012 [13]. Among people living with HIV, the proportion is even higher. For example, several Canadian studies have consistently found that more than half of HIV infected participants experienced FI [24,27,107].

FI is associated with a number of potential health problems, including poorer self-rated health, hypertension and depression, among the general population [42,108-110]. Among people living with HIV, FI may have additional HIV-related consequences, such as poor adherence to combined antiretroviral treatment (cART), poor HIV virological control, and poor immune recovery when they have FI [26,75,76,91]. In addition, risky sexual behavior and injection drug use (IDU) behavior are also more likely to occur when people experience FI [9,34,35]. While poor controlled HIV and poor immune recovery compromise individual health status, subsequent increases in community viral load [111] and the presence of risky sexual and injection behaviors [9,35] will facilitate HIV transmission. Therefore, FI may pose challenges to both individuals and public health when it occurs among people who are living with chronic infectious diseases, such as HIV.

As a consequence of similar routes of infection, hepatitis C virus (HCV) infection is common among HIV infected people [19], and with the advent of cART, HCV related liver disease became the main cause of mortality among HIV-HCV co-infected people [112,113].

However, recent empirical evidences have shown that the prevalence of HCV infection is declining in developed countries as a result of the reduction in new infections [114-116]. The recent introduction of more effective and tolerable HCV therapies, namely direct-acting antivirals (DAA), may further contribute to the declining trend of HCV infection in the long term [117]. Nevertheless, the current prevalence of HCV infection among HIV infected people is up to 15-30% in North America [118,119], so the HIV-HCV co-infected people are an important population subgroup. While co-infected people share many important characteristics with those who are HIV mono-infected, some additional characteristics may make this group particularly vulnerable. These include higher rates of IDU, levels of hepatic fibrosis and occurrence of chronic hepatotoxicity related to cART [120].

Although several prior studies have assessed the association between FI, HIV viral load and CD4 count [26,75,76,79,91,121], we are not aware of work assessing this association exclusively among HIV-HCV co-infected people. In addition, the majority of published studies on this association were cross sectional in nature and the temporal relationships between exposure and outcome were unclear. This problem is exacerbated when the association between exposure and outcome is bidirectional, such as the association between FI and HIV related health outcomes [122]. Furthermore, while cohort studies can better account for temporality, the appropriate use of analytical tools is necessary to estimate meaningful and unbiased associations [103]. For example, in a repeatedly measured cohort study, current HIV viral load and CD4 count are likely time-varying confounders. While adjusting for its confounding effect through conventional regression methods will introduce collider

stratification bias [123], failing to adjust for it will confound subsequent exposure-outcome associations.

Marginal structural models (MSMs) allow for the unbiased estimation of effects in the presence of time-varying confounders affected by prior exposure [103,124,125]. In this study, we examined the association between FI, HIV viral load, and CD4 count among HIV-HCV co-infected people participating in a Canadian cohort study using marginal structural model.

Methods

Study Population

We used data from the Food Security & HIV-HCV Sub-Study (FS sub-study) of the Canadian Co-infection Cohort (CCC) [126]. The CCC, initiated in 2003, is a nationwide prospective cohort study that includes 18 treatment sites across Canada and collects data on HIV-HCV co-infected people every six months [120]. Data on sociodemographic, socioeconomic, behavioral, clinical, and laboratory information are collected at each study visit. The FS sub-study was a mixed-methods cohort study initiated within the CCC in 2012 [107,126]. All CCC participants were invited to enroll in the FS sub-study and data on FI, depression, health care utilization and medication adherence were collected at the same CCC visit. Both the FS sub-study and the CCC were approved by the research ethics boards of the participating institutions [120], the secondary use of data in this study was approved by the McGill University Health Centre Research Ethics Board.

Exposure Measurement (Food Insecurity)

FI was measured using the adult scale of the Canadian Community Health Survey's (CCHS) Household Food Security Survey Module (HFSSM) [127] (a 10-item questionnaire); the HFSSM is based on the validated US Food Security Survey Module [2]. While the CCHS questionnaire measures FI over the past 12 months [127], the FS Study measured FI status over the past 6 months; this was done to synchronize the FS sub-study data collection procedure with the CCC [120]. In accordance with the CCHS categorization [127], FI was classified into three categories: food security (0 or 1 affirmative answer on the HFSSM), moderate food insecurity (2-5 affirmative answers) and severe food insecurity (≥6 affirmative answers).

Outcome Measurement (HIV Viral Load and CD4 Cell Count)

Both CD4 count (cells/uL) and HIV viral load (copies/mL) were measured once every six months based on the CCC protocol [120]. As current treatment guidelines recommend initiation of cART regardless of CD4 count [128,129], this continuous measure was not classified into categories. Instead, a natural log transformed CD4 count was used as a continuous outcome variable. On the other hand, an HIV viral load below <50 copies/mL is often used as a measure of treatment success [130]. Therefore, HIV viral load was classified as detectable (≥50 copies/mL) and undetectable (<50 copies/mL).

Covariate Measurement

Data for age (years), sex (male vs. female), marital status (married or having a common law partner vs. single, widowed, divorced), race (white, aboriginal or other), education level (less than high school, high school or some college), income (below 1000, 1001-3000 or above 3000 Canadian Dollars per month) and IDU in the past 6 months (yes vs. no) were collected

biannually through self-reports in questionnaires. Depression status was measured using the short version of the Center for Epidemiologic Studies Depression Scale (CES-D-10) [131]. This is a 10-item questionnaire assessing depressive symptoms in the past week. Each item has a four-point continuum from 0 to 3 and total score ranges from 0 to 30. Depression status was classified as absence of depressive symptoms if the total score was ≤ 10 and presence of depressive symptoms if it was > 10. Adherence was measured using the AIDS Clinical Trials Group (ACTG) Adherence Questionnaire [132], but we used the question "If you have missed doses during past 4 days, how many days have you missed all doses?" to define adherence level following prior literature [133]. Adherence was classified as perfect if there was zero reported missing day or imperfect otherwise.

Multiple Imputations

Although the maximum number of missing values of any given variable was low (see Table 3.2.1), this problem is compounded as the number of variables included in the analysis and the number of follow-up visits increases. Therefore, multiple imputation by chained equations [134] was used to impute missing values using 20 imputations with 100 iterations. Continuous variables were imputed by predictive mean matching; binomial variables were imputed using logit link; unordered categorical variables such as race were imputed with mlogit link (multinomial logistic link); ordered categorical variables such as educational level was imputed with ologit link (ordinal logistic link) [135].

Statistical Analysis

To account for potential time-varying confounders affected by prior exposure, stabilized inverse probability of treatment weights (SWs) were calculated by arranging the data set in long format (one person-visit per row) [103,124,136]. Sex, education, marital status, and IDU were used as time invariant confounders, while depression, HIV viral load and restricted cubic spline for natural log transformed CD4 count measured at the prior visit were used as time-varying confounders (See Appendix 3.2.1 for an assumed directed acyclic graph and Appendix 3.2.2 for the stabilized weight calculation). SW weighted outcome models, which are equivalent to marginal structural models, were fitted with the robust sandwich variance estimator to account for within subject clustering induced by repeated measurement and weighting. Specifically, a weighted linear regression model was used for the association between FI and log transformed CD4 count, and a Poisson regression model with log link was used for the association between FI and detectable HIV viral load [137,138]. The final point estimates and variances were combined across 20 imputed datasets using Rubin's rule [139].

In addition, a series of sensitivity analyses were conducted to assess the robustness of the association and potential collider stratification bias introduced by conditioning on time-varying confounders [123,140]. These were as follows,

1. A treatment indicator variable (on cART or not on cART) was included in the weight model as a baseline confounder in addition to existing variables used for the SW calculation. We assumed that HIV viral load and CD4 count are downstream variables relative to treatment indicator variable, and inclusion of HIV viral load and CD4 count as time-varying confounders should account for confounding effect of the treatment indicator variable.

2. The study sample was restricted to those who were on treatment (125 person-visits were excluded from a total of 1973 person-visits), and cART adherence measured at the prior visit was included in the weight model as a time-varying confounder in addition to HIV viral load and CD4 count with the same assumption as in sensitivity analysis 1.

3. With the same study sample as above, we used cART adherence measured at the prior visit as a time-varying confounder and excluded both HIV viral load and CD4 count from the weight model. In this analysis, we aimed to test if self-reported cART adherence is a good representation of actual adherence. We hypothesized that self-reported cART adherence should be able to capture all information included in HIV viral load and CD4 count if it represents the patients' actual adherence level. The result from this sensitivity analysis should be similar to that from the main analysis if the above hypothesis is correct.

4. Time-varying confounders along with baseline confounders were accounted for through standard regression adjustment to assess the potential impact of inappropriate adjustment of time-varying confounders on the exposure-outcome association.

In order to assess if a longer duration of FI has a more severe impact on HIV viral load and CD4 count, any FI that occurred at two or more consecutive study visits was defined as exposed. In this dichotomous exposure setting, the same potential confounders as in the main analysis were used. In addition, the exposure was lagged by one visit to assure that the outcome corresponds to the newly defined exposure.

Results

HIV-HCV co-infected patients were enrolled in this study from November 15, 2012 to October 15, 2015 and followed every 6 months for a maximum of five study visits. The number of patients completing each study visit were: 725, 608, 420, 203 and 17, respectively, contributing 1973 person-visits in total. At the baseline visit, 57% of patients experienced moderate or severe food insecurity and this percentage remained at around 50% throughout follow up. The proportions of patients with undetectable HIV viral load were 75%, 79%, 82%, 87% and 88% at each study visit and the median CD4 counts were 460, 480, 499, 532 and 530, respectively. The proportions of patients receiving HCV treatment at each study visit were 2.2%, 4.6%, 2.9%, 5.4% and 11.8%, respectively, and the proportions achieved undetectable HCV viral load 3 months after discontinuation of HCV treatment ranged from 44% to 60% throughout the study visits. Liver disease severity measured with Aspartate Aminotransferase to Platelet Ratio Index (APRI) varied from 0.59 (IQR: 0.37, 1.17) to 0.46 (IQR: 0.32, 1.07) across visits. Important sociodemographic, socioeconomic and clinical characteristics of study participants at baseline are presented in Table 3.2.1.

The main study results are presented in Table 3.2.2. Compared with people with no FI, people experiencing severe FI had 1.47 (95% CI: 1.14, 1.88) times the risk of having a detectable HIV viral load, and they had a 0.91-fold (95% CI: 0.84, 0.98) increase in CD4 cell counts on average (in other words, they had 9% fewer CD4 cells than people who were food secure). Moderate FI, however, did not have a statistically significant impact on either HIV viral load or CD4 count, although the point estimates for these associations were consistent with those found for severe FI. The SW distribution used for this analysis is displayed in Figure 3.2.1, and was stable throughout follow-up.

In the sensitivity analyses: 1) inclusion of a treatment indicator variable as an additional baseline confounder; 2) inclusion of cART adherence measured at a prior visit as a time-varying confounder; and 3) inclusion of cART adherence measured at a prior visit as a time-varying confounder by excluding both HIV viral load and CD4 count measured at the prior visit from the weight model did not change the main results meaningfully (sensitivity analysis 1, 2 and 3 in Table 3.2.3). In the last model (sensitivity analysis 4 in Table 3.2.3), where all baseline and time-varying confounders were adjusted through regression conditioning, only the association between severe food insecurity and detectable HIV viral load remained significant, and the point estimate decreased by 28% (calculated as (log(1.47)-log(1.32))/log(1.47)).

Table 3.2.4 presents results from analyses that used a slightly different exposure classification. When the exposure was defined as having FI (any level) at two or more consecutive visits, the associations both for HIV viral load and CD4 count were equivalent to the association for severe food insecurity presented in Table 3.2.2.

Discussion

We assessed the association between FI and both HIV viral load and CD4 count, among HIV-HCV co-infected people in Canada using prospectively collected data from the FS sub-study of the CCC [120,126]. The results indicated that severe FI was associated with increased risk of having detectable HIV viral load and lower CD4 count among HIV-HCV co-infected individuals. Our findings are consistent with the results of recent meta-analyses that summarized associations between FI and both HIV viral load and CD4 count among HIV infected people with an unknown proportion of HCV co-infection [141,142], and provide evidence that FI is a risk

factor for poor treatment outcomes among people co-infected with HIV-HCV. While the potential pathways through which FI affects HIV viral suppression and immune reconstitution remain unknown, it is very likely that poor medication adherence mediates the effect. Indeed, prior studies among HIV infected people have suggested this pathway [75,91]. Suboptimal adherence leads to HIV drug resistance [143], jeopardizes immune reconstitution and accelerates progression to AIDS. In addition, it may substantially change drug resistance profiles in the community if patients are already on second line and third line treatment regimens.

Since the introduction of cART, HCV related liver disease has emerged as a leading non-HIV cause of death among HIV-HCV co-infected people [112,113,144]. A French study documented a steady increase in the proportion of liver related deaths from 1995 to 2001 [145]. Recently, however, there have been major advancements in the treatment of HCV infection with the introduction of DAAs [117,146]. This may significantly improve the rate of sustained virologic response (SVR) and associated HCV-related morbidity and mortality. Although DAAs have superior effectiveness in HCV treatment, adherence is required to achieve SVR. Therefore, FI associated suboptimal adherence may compromise their beneficial effects. In other words, HIV-HCV co-infected people may not benefit from this new class of treatment if they experience FI. A recent study in Sub-Saharan Africa showed that food supplementation can increase treatment adherence [147]. Given the high proportion of FI among this population, food supplementation, therefore, may be a viable option to improve treatment adherence and subsequent health outcomes.

In the sensitivity analyses, when a baseline treatment indicator and cART adherence at prior visit were added separately to weight models, the original association obtained in the

main analysis were not altered (sensitivity analysis 1 and 2 in Table 3.2.3). This is an indication that the assumption regarding the causal structure in Appendix 3.2.1 is upheld. In other words, once the confounding effect of HIV viral load and CD4 count are taken into account, both treatment indicator and cART adherence become irrelevant. In sensitivity analysis 3, inclusion of cART adherence as a time-varying confounder and exclusion of both HIV viral load and CD4 count from the weight model produced similar results to the main model. This suggests self-reported adherence may represent actual adherence level and capture the information included in HIV viral load and CD4 count. In the last model, all associations shifted towards the null and the one that remained significant was also substantially attenuated. This may be attributable to collider stratification bias introduced by conditioning on potential time-varying confounders [123]. Our intention for the last sensitivity analysis was to show how the inappropriate use of analytical methods may bias the results.

When any FI that occurred at two or more consecutive study visits was defined as the exposure, the association between FI and both HIV viral load and CD4 count significantly strengthened and became equivalent to that seen with severe FI. This result suggests that sustained FI of any level that lasted for two or more consecutive visits had a similar effect on HIV viral load and CD4 count to severe food insecurity that occurred only once. In order to clarify if this dose response relationship was mainly driven by severe FI after collapsing moderate and severe FI into one category, we empirically examined the proportion of FI level at each study visit and found that the proportion ranged from 20% to 35% for moderate FI and 25% to 35% for severe FI. Therefore, the result of this analysis is unlikely to be driven only by severe FI.

This study has some unique features and strengths. First, this is a cohort study with a clear temporal sequence between variables measured at multiple visits. Second, as it is possible to have time-varying confounders in a repeatedly measured cohort study, an epidemiological method specifically designed to tackle this problem was applied to obtain association measures. Thirdly, the study population is comprised exclusively of HIV-HCV co-infected subjects. HIV-HCV co-infected people tend to have significantly higher mortality compared with HIV mono-infected individuals [148]. As uncontrolled HIV infection is probably associated with this excess mortality [144], identifying potential factors associated with poor HIV viral control among HIV-HCV co-infected people may provide some insights for health professionals. However, there are also several limitations. For example, we cannot claim a causal relationship between FI, HIV viral suppression and CD4 count even though we applied causal inference methodology. First, in order to have a causal association, an unverifiable assumption such as no unmeasured confounder needs to be held [103]. Although we believe that the most important confounders were included in the analysis, given the complex psychosocial characteristics involved in FI, there may be potential confounders that were not included in the model. Secondly, FI may not correspond to a well-defined intervention. There are different reasons for a patient to experience FI [24,107] and each reason may have different implications for having detectable HIV viral load and lower CD4 count. In other words, the necessary condition for a causal inference, the counterfactual consistency assumption, may not hold for this exposure [149].

In summary, we found that severe FI is associated with detectable HIV viral load and lower CD4 count among HIV-HCV co-infected people. Additionally, there is a clear dose

response relationship in terms of severity and duration of FI. Addressing FI among people co-infected with HIV-HCV may improve HIV related health outcomes.

Table 3.2.1 Baseline characteristics by food insecurity status of the Food Security & HIV-HCV Sub-study participants, Canada, 2012-2015

Characteristics	Total (N=725)	Food secure (N=309)	Food Insecure (N=416)		No. of missing values for each variable
			Moderate (N=170)	Severe (N=246)	
Age (years)[a]	49 (43, 54)	50 (44, 55)	48 (40, 53)	48 (43, 53)	2
Male[b]	528 (73%)	240 (78%)	114 (67%)	174 (71%)	4
Caucasian	542 (75%)	237 (77%)	119 (70%)	186 (76%)	10
Married or common law	133 (18%)	67 (22%)	28 (16%)	38 (15%)	12
College or above education	190 (26%)	106 (34%)	27 (16 %)	57 (23%)	9
Monthly income below 1000 CAD [c]	308 (42%)	106 (34%)	72 (42%)	130 (53%)	6
IDU in the past 6 months	238 (33%)	60 (19%)	62 (36%)	116 (47%)	21
Depressive symptoms	338 (47%)	89 (29%)	81 (48%)	168 (68%)	8
Clinical AIDS	43 (6%)	20 (6%)	6 (4%)	17 (7%)	2
On cARV	662 (91%)	289 (94%)	160 (94%)	213 (87%)	0
HIV infection duration (years) [a]	14.1 (8.3, 18.4)	15.3 (8.3, 20.0)	13.1 (8.5, 17.1)	13.5 (8.2, 17.4)	24
CD4 cell count below 350 cell/uL	216 (30%)	78 (25%)	52 (31%)	86 (35%)	31
CD4 cell count (cell/uL) [a]	460 (300, 665)	491 (320, 692)	457 (290, 645)	440 (288, 630)	31
HIV RNA below 50 copies/mL	547 (75%)	248 (80%)	127 (75%)	172 (70%)	44
Natural log of HIV RNA	4.3 (2.1)	4.1 (1.9)	4.3 (1.9)	4.6 (2.4)	44
On HCV treatment	16 (2%)	9 (3%)	4 (2%)	3 (1%)	1
Undetectable HCV viral load [d]	142 (59%)	74 (59%)	25 (64%)	43 (60%)	39
APRI [a e]	0.54 (0.35, 1.07)	0.59 (0.38, 1.2)	0.53 (0.34, 1.13)	0.62 (0.34, 1.16)	56

a, Median (Inter Quartile Range); b, Number (Proportion); c, Canadian Dollar; d, Number (Proportion) of patients achieved undetectable HCV viral 3 months after discontinuing HCV treatment; e, Aspartate Aminotransferase (AST) to Platelet Ratio Index.

Table 3.2.2 Association between food insecurity and both HIV viral load and CD4 count among the Food Security & HIV-HCV Sub-study participants, Canada, 2012-2015

Associations	Food Insecurity		Stabilized Weight	
	Moderate	Severe	Mean (SD)[a]	Min/Max[b]
Relative Risk for Detectable HIV RNA	1.15 (0.88, 1.51)	1.47 (1.14, 1.88)	1.000 (0.121)	0.257/2.380
Fold Increase in CD4 Count	0.94 (0.87, 1.02)	0.91 (0.84, 0.98)		

a, Mean and Standard Deviation (SD) was calculated individually for each imputed data set and then combined using Rubin's rule [139]; b, The minimum and maximum values were based on the entire imputed data set.

Table 3.2.3 Association between food insecurity and both HIV viral load and CD4 count under different confounder combination, lagging and regression adjustments for time-varying confounders among the Food Security & HIV-HCV Sub-study participants, Canada, 2012-2015

Sensitivity Analysis	Relative Risk for Detectable HIV RNA		Fold Increase in CD4 Count	
	Moderate FI	Severe FI	Moderate FI	Severe FI
1	1.13 (0.87, 1.48)	1.37 (1.07, 1.76)	0.95 (0.87, 1.02)	0.91 (0.85, 0.98)
2	1.13 (0.83, 1.53)	1.42 (1.07, 1.89)	0.94 (0.87, 1.02)	0.91 (0.84, 0.99)
3	1.19 (0.88, 1.61)	1.38 (1.04, 1.84)	0.94 (0.86, 1.01)	0.91 (0.84, 0.98)
4	1.10 (0.87, 1.38)	1.32 (1.05, 1.67)	1.0 (0.96, 1.05)	0.98 (0.93, 1.04)

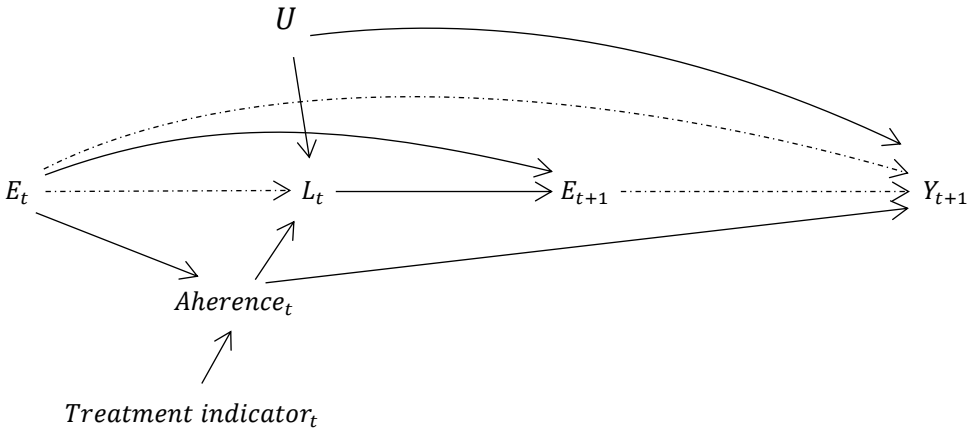
Table 3.2.4 Association between food insecurity and both HIV viral load and CD4 count when exposure was defined as two or more consecutive food insecurity among the Food Security & HIV-HCV Sub-study participants, Canada, 2012-2015

Exposure	Relative Risk for Detectable HIV RNA	Fold Increase in CD4 Count	Number of Person-visits contributed [a]
Any FI at two or more consecutive visits	1.46 (1.07, 1.98)	0.90 (0.83, 0.97)	1142

a, Total number of person-visits is 1973

Appendix 3.2.1 Assumed Causal Structure between Variables

When analyzing the association between FI and both HIV viral load and CD4 count using data from a repeatedly measured cohort study, the HIV viral load and CD4 count (outcome variables) measured at prior visit will act as time-varying confounders affected by prior exposure for the subsequent association. Consider the following directed acyclic graph (DAG) (baseline confounders are ignored) [150]



E_t is food insecurity status measured at visit t and because of the nature of the questionnaire, it reflects participant's food insecurity experience over the past 6 months. $Adherence_t$ is adherence level measured at visit t. $Treatment\ indicator_t$ is a variable that indicates whether a patient was on cART or not at visit t. L_t is HIV viral load and CD4 count measured at visit t and Y_{t+1} is HIV viral load and CD4 count measured at visit t+1. U includes unmeasured variables that affect patient's immune status such as genetic factors, exercise level, health awareness, etc. Dashed arrow represents the association between FI and HIV viral load (and CD4 count). Low CD4 count and high HIV viral load may affect patient's food insecurity status measured at subsequent visit (represented with the arrow from L_t to E_{t+1}), because weak immune status at visit t may prevent patients from productive activities and may make them prone to have food

insecurity at visit t+1. Weak immune status at visit t is also a predictor of immune status at visit t+1. In addition, it was shown that HIV viral load and CD4 count is affected by food insecurity among HIV infected people [75,141,142] (represented with the dashed arrow from E_t to L_t). Therefore, HIV viral load and CD4 count measured at visit t (L_t in the DAG) can be time-varying confounders for the association between food insecurity measured at visit t+1 (E_{t+1} in the DAG), and HIV viral load and CD4 count measured at visit t+1 (Y_{t+1} in the DAG).

$Adherence_t$ is a predictor of HIV viral load and CD4 count (the arrow from $Adherence_t$ to L_t and from $Adherence_t$ to Y_{t+1}). It also affects E_{t+1} through worsened health status (represented with the arrows $Adherence_t \rightarrow L_t \rightarrow E_{t+1}$). $Adherence_t$ is likely affected by prior exposure status (E_t) [75] (although they were measured at the same visit, we assumed that the time window in which they were measured assures temporality between them). Therefore, $Adherence_t$ is a time-varying confounder effected by prior exposure.

Only patients who started cART can be evaluated on adherence. Therefore, $Treatment\ Indicator_t$ is upstream variable relative to $Adherence_t$. However, $Treatment\ Indicator_t$ is not likely affected by E_t. In other words, physicians make their decision on cART initiation based on clinical consideration rather than patients' food insecurity status.

As the potential confounding effect of $Treatment\ indicator_t$ will act through L_t (HIV viral load and CD4 count), inclusion of L_t in the weight calculation should be enough to control for the confounding effect of $Treatment\ indicator_t$. In the sensitivity analysis 1,

$Treatment\ indicator_t$ was included in the weight model in addition to L_t to assess if this alters the exposure-outcome association.

$Adherence_t$ is also an upstream variable relative to L_t. Therefore, inclusion of L_t in the weight model should be enough to account for the confounding effect of $Adherence_t$. In the sensitivity analysis 2, $Adherence_t$ was added as a time-varying confounder in addition to L_t to assess if this meaningfully alters the exposure-outcome association.

If self-reported $Adherence_t$ represents actual adherence level, it should capture information included in L_t and inclusion of $Adherence_t$ in the weight model should be sufficient to account for the confounding effect of L_t. In the sensitivity analysis 3, $Adherence_t$ was included in the model by excluding L_t to assess if the results will be similar to that obtained from the main analysis. Obtaining similar results to the main analysis means self-reported adherence can approximate actual adherence.

Appendix 3.2.2 Calculation of Stabilized Weights (SW)

SW was calculated using the following formula [103,124,136]

$$SW_{ik}^{E} = \frac{\Pr[E_{ik}|E_{ik-1},V_{io},\bar{C}_{jk}=0]}{\Pr[E_{ik}|E_{ik-1},L_{ik-1},\bar{C}_{jk}=0]}$$

E_{ik} represents participant i's FI status at visit k and it measures patient's food experience over the past 6 months. V_{i0} includes baseline confounders (sex, education level, marital status, IDU in the past 6 months). C_{jk} is censoring indicator. L_{ik-1} includes both time-varying and baseline confounders.

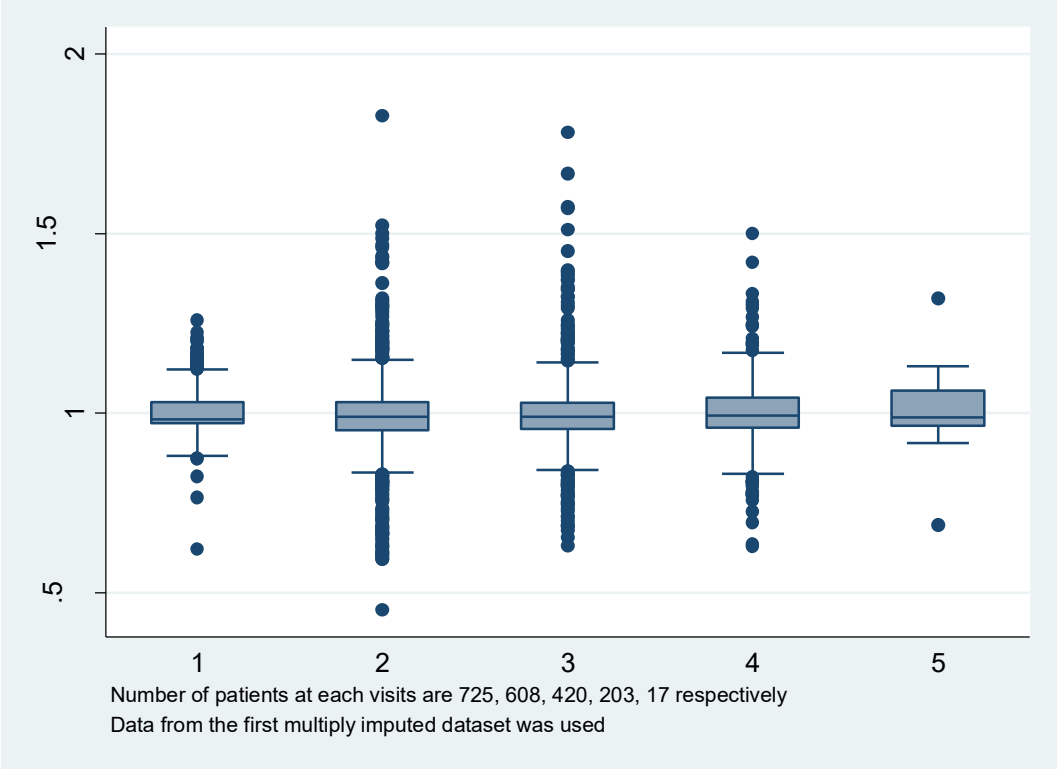
It should be noted that the above formula does not have cumulative multiplication mark even though it was suggested in the literature [103,124,136]. Because our interest was to assess the effect of FI measured at visit k (food experience over the past 6 months) on HIV viral suppression and CD4 count measured at visit k rather than to assess the effect of specific pattern of exposure (e.g. exposed at all visits vs. unexposed at all visits). Therefore, the result from weighted outcome model (weighted with person-visit specific weights) is a comparison of HIV viral load and CD4 count when all study participants were exposed over the past 6 months vs. all study participants were not exposed over the past 6 months. Although both exposure and outcome were measured at the same visit, the HFSSM questionnaire measured patient's food experience over the past 6 months, whereas HIV viral load and CD4 count measured at the same visit likely reflect their current levels.

In the calculation of the exposure probabilities at baseline visit, we need to use unmeasured values, $E_{i,-1}$ and $L_{i,-1}$. As cohort data collection does not correspond to any

conceptual time zero, it is impossible to simply set $E_{i,-1}$ and $L_{i,-1}$ to any specific values. In other words, people could be exposed or unexposed at potential visit that could have occurred before cohort entry. One possible way to address this issue is to set both $E_{i,-1}$ and $L_{i,-1}$ to their corresponding baseline values. For example, patients with FI at baseline were also more likely to be FI at potential visit that could have occurred before baseline. However, food insecurity (included in $E_{i,-1}$), HIV viral load (included in $L_{i,-1}$), CD4 count (included in $L_{i,-1}$) and depression status (included in $L_{i,-1}$) are likely multifactorial and taking other factors into account may increase the chance of 'filling in' their values correctly. Therefore, $E_{i,-1}$ and $L_{i,-1}$ were predicted by arranging data set in long format and using measured FI, sex, education level, marital status, IDU in the past 6 months, depression, HIV viral load, CD4 count. By arranging the data set in long format, the strength of association between $E_{i,k}$ and $E_{i,k+1}$ within cells defined by $V_{i,0}$ and $L_{i,k}$ can be utilized to 'fill in' the most probable values of $E_{i,-1}$; and the strength of association between $L_{i,k}$ and $L_{i,k+1}$ within cells defined by $V_{i,0}$ and $E_{i,k}$ can also be utilized to 'fill in' the most probable values of $L_{i,-1}$.

The exposure variable has three categories: food security, moderate food insecurity and severe food insecurity. Therefore, multinomial regression model was fitted to predict exposure probabilities both in the numerator and denominator in the weight model [103].

Figure 3.2.1 Stabilized weight distribution across study visits among the Food Security & HIV-HCV Sub-study participants, Canada, 2012-2015



Chapter 4: Assessing the Association between Exposure and Mediator

4.1 Preface to Manuscript 4

In order to quantify the direct effect of exposure on outcome that is not mediated through a hypothesized mediator, the first step is to assess if that hypothesized mediator is on the pathway between exposure and outcome. In other words, it should be first shown that the exposure is associated with the hypothesized mediator and second that the hypothesized mediator is associated with the outcome. In this thesis, we hypothesized that depressive symptoms mediate part of FI effects on HIV viral load and CD4 count among HIV-HCV co-infected people. In chapter 3, it was shown that FI is associated with both poor HIV viral control and lower CD4 count in this population. In this chapter, the impact of FI on the occurrence of depressive symptoms was assessed.

Depressive symptoms are also shown to be associated with an array of behavioral and health outcomes, including: poor cART adherence, risky sexual behavior, and higher mortality. As such, depressive symptoms not only contribute to poor health status, it may also facilitate secondary HIV transmission through increased risk taking behaviours and higher community viral load. With these negative health outcomes in mind, identifying risk factors for developing depressive symptoms is also important for improving individual and public health outcomes. Because the development of depressive symptoms is multifactorial and complex process in nature, other factors such as living with HIV, social stigma and unstable housing may also play important roles. Therefore, identification of FI as yet another additional risk factor for depressive symptoms does not necessarily mean that intervention on FI would improve

depressive symptoms. Due to the multi factorial nature of depressive symptoms, intervention strategies must consider a variety of other factors. For example, psychosocial assessment and support could mitigate the harmful impact of FI among HIV-HCV co-infected people.

To our knowledge, there is no previous study that assessed this association exclusively among people co-infected with HIV-HCV. Thus, this study may provide important insights regarding the role of FI in the occurrence of depressive symptoms among co-infected people.

4.2 Manuscript 4

Impact of Food Insecurity on Depressive Symptoms Among HIV-HCV Co-Infected People

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Publication status: AIDS and Behavior, *In press*

Abstract

Food insecurity (FI) is associated with depressive symptoms among HIV mono-infected people. Our objective was to examine whether this association holds among HIV- hepatitis C virus (HCV) co-infected people. We used data from a prospective cohort study of HIV-HCV co-infected people in Canada. FI was measured using the 10-item adult scale of Health Canada's Household Food Security Survey Module (HFSSM) and was classified into three categories: food secure, moderate FI, and severe FI. Depressive symptoms were measured using the Center for Epidemiologic Studies Depression Scale (CES-D-10) and was classified into absence or presence of depressive symptoms. FI, depressive symptoms, and other covariates were updated every 6 months. The association between FI and depressive symptoms was assessed using a stabilized inverse probability weighted marginal structural model. The study sample included 725 HIV-HCV co-infected people with 1973 person-visits over 3 years of follow up. At baseline, 23% of participants experienced moderate food insecurity, 34% experienced severe food insecurity and 52% had depressive symptoms. People experiencing moderate FI had 1.63 times (95% CI 1.44-1.86) the risk of having depressive symptoms and people experiencing severe FI had 2.01 times (95% CI 1.79-2.25) the risk of having depressive symptoms compared to people who were food secure. FI is a risk factor for developing depressive symptoms among HIV-HCV co-infected people. Food supplementation, psychosocial support and counseling may improve patient health outcomes.

Keywords: HIV-HCV co-infection; food insecurity; depression; marginal structural model.

Introduction

Introduction of combined antiretroviral treatment (cART) greatly increased the life expectancy of HIV infected people and rendered HIV infection a chronic manageable condition in developed countries [21,151,152]. A Canadian study estimated that up to one third of the HIV infected adult men and women receiving care in the province of Ontario had depressive symptoms [153] and similar estimates have been reported in the United States [39]. Studies also indicated that people experiencing depressive symptoms tended to have poor cART adherence, poor virologic control, risky sexual behavior, and higher mortality [44-47]. As such, depression not only exacerbates individual health status, it may also facilitate secondary HIV transmission through increased risk taking behaviours and higher community viral load [111].

Development of depression among HIV infected people is a multifactorial and complex process in which factors such as the diagnosis of HIV, social stigma [41], loss of social and familial support [154,155], and unstable housing may play important roles [156]. Recently, food insecurity (FI), defined as a limited or uncertain ability to acquire acceptable foods in socially acceptable ways, or limited or uncertain availability of nutritionally adequate and safe foods [106], has also been identified as an important factor associated with depressive symptoms among this population [32,42,43,157]. FI is of particular concern given its high prevalence among HIV infected people [24,42,107,158] and strong association with depression [42,157,159].

While the association between FI and depression has previously been assessed among HIV infected people [24,32,42,157], no prior study assessing this association exclusively among

HIV-HCV co-infected people. Due to shared routes of transmission, HCV infection is common among HIV infected people [19,21,119]. In Canada, there were an estimated 13,000 people with HIV-HCV co-infection in 2008, accounting for about 20% of total HIV infected individuals in that year [21]. Since the majority of HCV infection among HIV positive people may become chronic as apposed to those among HIV negative people [20], the total number of HIV-HCV co-infection may further increase with the increased life expectancy of HIV infected individuals [151,152]. Therefore, it is increasingly important and urgent to address public health problems among this population subgroup.

Although it is tempting to extrapolate findings from people with HIV mono-infection to those with HIV-HCV co-infection, the latter group has certain unique characteristics that may prevent such extrapolation. Indeed, HCV co-infection makes HIV infected people more vulnerable to develop depression, due in part to HCV's activity in the central nervous system [160], side effects of HCV treatment [161,162], and higher rates of injection drug use (IDU) [163,164]. Therefore, it is unclear to what extent the association between FI and depressive symptoms holds among HIV-HCV co-infected people. In this study, we assessed the association between FI and depressive symptoms exclusively among HIV-HCV co-infected adult men and women using prospectively collected data in Canada.

Method

Study sample

This study used data from the Food Security & HIV-HCV Sub-Study (FS Sub-Study) of the Canadian Co-Infection Cohort (CCC) [120,165]. The recruitment process, measurement of

variables, and follow up procedure of the CCC study has been previously described [120]. Briefly, the CCC study is a prospective cohort study that was initiated in 2003 and recruited HIV-HCV co-infected people aged>16 years from both major urban centres and smaller cities to reflect the Canadian epidemic. The CCC includes 18 treatment sites across Canada and updates sociodemographic, behavioral, clinical and laboratory information every six months. The FS Sub-Study was initiated in 2012 within the CCC [165]. All CCC participants were invited to participate in the FS Sub-Study and participants provided additional information on FI, depression, health care utilization, and medication adherence at each visit. As in the CCC, information in the FS Sub-Study was collected biannually using questionnaires [165]. Both the FS Sub-Study and the CCC were approved by the research ethics boards of the participating institutions [120], and the secondary use of data for this analysis was approved by the McGill University Health Centre Research Ethics Board.

Food Insecurity (exposure)

FI was measured using the 10-item adult scale of Health Canada's Household Food Security Survey Module (HFSSM) [166]. In this study, the HFSSM measured participants' FI experience over the past 6 months and FI status was classified based on the number of affirmative answers on to the HFSSM. An answer is considered affirmative if it is either "often true" or "sometimes true" to questions such as "Since your last visit, did you ever cut the size of your meals or skip meals because there wasn't enough money for food?". In accordance with the Canadian Community Health Survey (CCHS) categorization [166], this variable was classified into three categories: food security (0 or 1 affirmative answer on the HFSSM), moderate food insecurity (2-5 affirmative answers), or severe food insecurity (≥6 affirmative answers).

Depression (outcome)

Depressive symptoms were measured using the Center for Epidemiologic Studies Depression Scale (CES-D-10) [131]. a 10-item questionnaire simplified from the standard 20-item scale [167]. The CES-D-10 was shown to have comparable predictive accuracy with the original scale [131] and has been validated in the Canadian context [168]. It includes questions about how often one has had symptoms related to depression over the past week, such as feeling blue, restless sleep, and loneliness. Each of the ten items has a four-point continuum from 0 to 3 and the total score ranges from 0 to 30. Since the cut-off score of $\geqslant$10 was shown to have the best combination of sensitivity and specificity in differentiating clinically depressed people [169], participants were considered to have depressive symptoms if their total score was ≥10 and normal otherwise.

Covariates

Sex, age, race, marital status, education level, income, and IDU in the past 6 months were updated at each visit. HIV clinical stage was classified into 9 categories based on the Centers for Disease Control and Prevention's (CDC) 1993 revised classification system for HIV infection [170]. Since more finely defined categories may not necessarily result in better control of confounder [124], the original 9 categories of clinical stage were collapsed into three (I=A1+A2+A3, II=B1+B2+B3, and III=C1+C2+C3) to achieve better model convergence.

Statistical analysis

Missing data were imputed using multiple imputations by chained equations [134] with 20 imputed datasets. To obtain an exposure effect with a population average ("marginal")

interpretation, stabilized inverse probability weights (SW) were used to account for potential time-varying confounders (Appendix 4.2.1) [103,124]. Confounders were selected a priori based on consultation with subject matter experts and the literature [42,107,171-173], and their inclusion in the models was guided by a directed acyclic graph (Appendix 4.2.2) [150]. Accordingly, sex, age, race, marital status, education level, income, and IDU in the past 6 months were used as baseline confounders; past HIV clinical stage and past depression status were used as time-varying confounders. A weighted Poisson regression model with log link was used to model the outcome [137]. A robust sandwich variance estimator was specified to account for within subject clustering induced by repeated measurements and weighting. The final point estimate and variances were combined across the 20 imputed datasets using Rubin's rules [139]. In addition, unweighted estimates (the crude association, baseline adjusted association, and baseline plus time-varying confounder adjusted association) were presented as a comparison with the weighted results. Since prevalence of depression may vary by sex [174] and educational level [175], subgroup analyses were conducted to explore whether effects differ in certain subgroups.

In order to assess the duration of FI's impact on depressive symptoms, we lagged the exposure variable by one visit, two visits, and three visits, respectively. In each of the lagged analyses, the impact of the index FI was assessed by blocking the impact of FI measured between index FI and the outcome variable using a causal mediation approach (Appendix 4.2.3) [176]. When the number of lagged visits increases, the person-visit contribution decreases, and this reduces statistical power. Therefore, moderate FI and severe FI were collapsed into one FI

category and participants experiencing any FI were compared with participants who were food secure.

Since the first item of the HFSSM scale measures if a participant *worried* about running out of food [166], it is possible that depressed persons provided affirmative answers to this item even when FI was not experienced. In other words, there may be reverse causation due to the psychometric property of the HFSSM. Therefore, a sensitivity analysis was conducted by reversing positive responses to this item to negative ones if people had any depressive symptoms at a given study visit.

Results

Sociodemographic, socioeconomic, behavioral, and clinical characteristics of participants at cohort entry are shown in Table 4.2.1. Briefly, a total of 725 HIV-HCV co-infected adult men and women were enrolled from November 15, 2012 to October 15, 2015 and followed every six months for a maximum of 5 study visits. Age was similar across exposure groups with an overall median age of 49 (IQR: 43-54). The sample was mostly male (73%), Caucasian (75%), and receiving cART (91%). Less than 20% were married or had a common-law partner, only 26% had some college education and one half had a gross income less than 1000 Canadian dollars (CAD) per month. One third had injected drugs in the past 6 months. The median duration of HIV infection was 14 years and 6% were at HIV clinical stage III. The prevalence of any FI was 57% at cohort entry, and half of study participants had depressive symptoms.

Table 4.2.2 displays results from unweighted and weighted generalized linear models. The unadjusted model indicated that compared to food secure participants, those experiencing moderate FI had 1.78 (95% CI 1.57-2.02) times the risk of having depressive symptoms; this association was stronger (RR=2.38, 95% CI 2.14-2.65) when participants experienced severe FI. After adjusting for baseline confounders, the results did not change meaningfully. Accounting for both baseline and time-varying confounders through regression adjustment attenuated the associations and reduced the strengths of the original associations by 50-60%. The final model, which accounting for potential time-varying confounders through weighting, indicated that participants who experienced moderate FI had 1.63 (95% CI 1.44, 1.86) times the risk and participants who experienced severe FI had 2.01 (95% CI 1.79-2.25) times the risk of developing depressive symptoms, compared to participants who were food secure. The 95% CIs of these two estimates barely overlap, possibly indicating a dose response relationship in terms of FI severity. The calculated SW used for this analysis ranged from 0.414 to 3.363 with a mean of 1.002 throughout study visits (Figure 4.2.1).

Results of the subgroup analyses are presented in the forest plot (Figure 4.2.2). Participants experiencing FI had a significantly greater risk of having depressive symptoms, compared to participants who were food secure in each of the subgroups (except the one in which people had some college education and experienced moderate FI). The dose response relationship in terms of severity of FI persisted regardless of subgroup although this was less obvious among people with less than a high school education. There was no sex difference observed within a given level of FI. The strength of association between moderate FI and

depressive symptoms deceases with the increase of educational level; this trend is weaker for severe FI.

When FI was lagged by one study visit and the impact of FI measured after the index FI was blocked using a causal mediation method [176], there was still a statistically significant association between FI and depression (RR=1.20, 95% CI 1.02-1.40) although there was substantial attenuation compared to the main result in Table 4.2.2. Experiencing FI appears to be protective after lagging the exposure variable by two study visits; the association disappeared after lagging three study visits (Table 4.2.3).

In the sensitivity analysis, where the affirmative response to the item that measures *worrying* about food was reversed if participants had any concomitant depressive symptoms, the original associations were attenuated but still statistically significant. Participants experiencing moderate FI were 1.44 (95% CI 1.27-1.62) times more likely to have depressive symptoms compared to people who were food secure, and the RR for severe FI was 1.72 (95% CI 1.54-1.91).

Discussion

Among our study population, approximately 50% of participants experienced FI at any time during follow up. This was consistent with prior findings among HIV mono-infected people from various settings [24,32,42,158]. The prevalence of depressive symptoms at any time during follow up, however, is considerably higher than the estimates among HIV mono-infected people. A Canadian based group estimated that 28% of their cohort of 3,816 HIV infected people reported depressive symptoms at baseline [153]. An earlier study on a representative

sample of HIV infected people in the United States reported a similar prevalence of major depression [39]. Although many factors, including the diagnosis of HIV, may contribute similarly to the development of depressive symptoms among HIV mono-infected and HIV-HCV co-infected people, certain additional factors may play an important role among the HIV-HCV co-infected population. For example, neurocognitive impairment caused by HCV's activity in the central nervous system [160], side effects from HCV treatment [161] and higher rates of IDU [163,164] may contribute to the development of depressive symptoms among HIV-HCV co-infected people.

To the best of our knowledge, this is the first prospective cohort study that assessed the temporal association between FI and depressive symptoms exclusively among HIV-HCV co-infected people. Our results indicated that both moderate and severe FI were significant risk factors for developing depressive symptoms among co-infected people. The association between severe FI and depressive symptoms was 40% stronger than the one found with moderate FI. However, these findings were conservative compared to a prior study among HIV infected people with unspecified HCV co-infection status in San Francisco, United States [42]. In this study, FI was measured using the Household Food Insecurity Access Scale (HFIAS) and depressive symptoms were ascertained using the Beck Depression Inventory (BDI-II) [42]. In addition, the association was reported as an odds ratio (OR) with a relatively common outcome. Due to a mathematical property of the OR, this measure tends to overestimate the association when the prevalence of outcome is common (e.g. >10%). These may explain the differences in the magnitude of the effect estimates. Another study among women in the United States who were at risk for HIV also documented a strong association between FI and depression [177]. In

this study, FI was measured with a similar scale as in our study, but with 4 items instead of 10, and depressive symptoms were measured with the 20-item CESD scale [167]. Again, the association was expressed with ORs. Nevertheless, these consistent findings from various settings may support the existence of a relatively strong association between FI and depressive symptoms.

In subgroup analyses, we were unable to find a differential effect of FI on depressive symptoms across sex and this result is consistent with the previous findings among HIV infected individuals in the United States [42]. Therefore, the strength of the association between FI and depressive symptoms may not vary across sex among HIV infected people, even though differential effects were found among general population [178,179]. When experiencing moderate FI, people with some college education did not develop depressive symptoms while people with less than high school education had 2 times greater risk of developing depressive symptoms. This is possibly because people with higher education may have better coping strategies [180]. In addition, better employment prospects may also help relieve emotional stress. However, these education level differences largely disappeared when people were experiencing severe FI. When facing immediate shortage of food, as indicated by severe FI, people with higher educational level appears as likely to develop depressive symptoms as those with lower educational level.

After lagging the exposure by one visit and blocking the mediating effect of exposure measured at current visit, people experiencing FI at prior visit still have a significantly higher risk for developing depressive symptoms at the current visit compared with people who were food secure at the prior visit. In the causal mediation literature, this is called a controlled direct

effect (CDE) [176] and it measures the effect of an exposure on an outcome after forcing intermediate variables (e.g. FI measured at current visit in this case) to take on the same value for all subjects. Under the assumption of no exposure-mediator interaction [181], the CDE estimated the index FI effect measured at a prior visit on depressive symptoms at the current visit when all subjects were either food insecure or food secure at the current visit. In other words, our results indicate that the effects of FI on depressive symptoms persist for 6-12 months even if people recovered from FI status at the current visit. Psychological stress that occurs when people experience FI may not fully explain this phenomenon (having depressive symptoms even after recovered from FI), and support the existence of other mechanisms. Indeed, different coping strategies induced by FI, such as reduced consumption of food, may lead to micronutrient deficiencies over the long term [60] and micronutrient deficiencies was shown to be associated with depression [182,183]. This long-term effect of FI on depressive symptoms may necessitate ongoing monitoring and psychosocial support even after recovery from FI. When the exposure was lagged by two visits, experiencing FI at index visit appears protective against developing depressive symptoms at current visit. While it is possible that experiencing certain stressful events may make some people become resilient [184], we wish to apply caution. Indeed, the person-visit contribution is nearly half of the first lagged analysis and the result from lagging exposure by three visits did not show a similar protective effect.

Our study has several strengths. First, this is a prospective cohort study that enabled a clear temporal sequence between exposure and outcome. Second, this cohort is comprised exclusively of HIV-HCV co-infected individuals. Therefore, the result may have better application to address public health concerns of this population subgroup. Third, appropriate

analytical methods were applied to account for potential time-varying confounders. Finally, a sensitivity analysis was conducted to assess the robustness of the results. However, there are also several limitations. First, it is difficult to claim a causal relationship between FI and depressive symptoms due to the observational nature of this study. Second, the outcome was self-reported depressive symptoms rather than clinical diagnoses. However, the CES-D-10 is a validated tool to be used in epidemiologic studies [168] and has acceptable sensitivity and specificity [169].

In summary, our results suggest that experiencing FI is a significant risk factor for developing depressive symptoms among HIV-HCV co-infected adult men and women. FI has a differential effect on depressive symptoms across people with different level of educational achievement, but there was no evidence of differential effect across sex. The negative impact of FI on depressive symptoms appears to persist even after recovering from FI. Therefore, psychosocial assessment and support may be needed as part of interventions to FI to mitigate its harmful impact among HIV-HCV co-infected people.

Table 4.2.1 Baseline characteristics by food insecurity status of the Food Security & HIV-HCV Sub-study participants, Canada, 2012-2015

Characteristics	Total (N=725)	Food secure (N=309)	Food Insecure (N=416)		Total No. of missing for each variables
			Moderate (N=170)	Severe (N=246)	
Age (years) [a]	49 (43, 54)	50 (44, 55)	48 (40, 53)	48 (43, 53)	2
Sex [b]	–	–	–	–	4
Male	528 (73)	240 (78)	114 (67)	174 (71)	–
Female	186 (26)	66 (21)	53 (31)	67 (27)	–
Transgender	7 (1)	2 (1)	2 (1)	3 (1)	–
Ethnicity [b]	–	–	–	–	10
White	542 (75)	237 (77)	119 (70)	186 (76)	–
Aboriginal	129 (18)	49 (16)	42 (25)	38 (15)	–
Asian, Black, Hispanic, Latino	44 (6)	22 (7)	5 (3)	17 (7)	–
Married or common law [b]	133 (18)	67 (22)	28 (16)	38 (15)	12
Sexual orientation [b]	–	–	–	–	9
Heterosexual	498 (67)	196 (63)	127 (75)	175 (71)	–
Homosexual	148 (20)	88 (28)	22 (13)	38 (15)	–
Bisexual	70 (10)	24 (8)	19 (11)	27 (11)	–
Education [b]	–	–	–	–	9
Less than high school	144 (20)	51 (17)	50 (29)	43 (17)	–
High school	382 (53)	152 (49)	88 (52)	142 (58)	–
College or university	190 (26)	106 (34)	27 (16)	57 (23)	–
Unstable housing [b c]	100 (14)	28 (9)	25 (15)	47 (19)	1
Monthly income [b]	–	–	–	–	6

<1000 CAD	308 (42)	106 (34)	72 (42)	130 (53)	–
1001-2999 CAD	347 (48)	142 (46)	92 (54)	113 (46)	–
>3000 CAD	64 (9)	57 (18)	6 (4)	1 (0.4)	–
IDU in the past 6 months [b]	238 (33)	60 (19)	62 (36)	116 (47)	21
Clinical stage [b]	–	–	–	–	2
Stage I	636 (88)	264 (85)	154 (91)	218 (89)	–
Stage II	44 (6)	24 (8)	10 (6)	10 (4)	–
Stage III	43 (6)	20 (6)	6 (4)	17 (7)	–
On cARV [b]	662 (91)	289 (94)	160 (94)	213 (87)	0
HIV infection duration (years)	14.1 (8.3, 18.4)	15.3 (8.3, 20.0)	13.1 (8.5, 17.1)	13.5 (8.2, 17.4)	24
Depressive symptoms [b d]	377 (52)	106 (34)	92 (54)	179 (73)	8

a, Median (Inter Quartile Range); b Number (Proportion); c, Unstable housing was defined by the occurrence of either one of these situations: not being a homeowner and not being a renter or not having a fixed address since last visit or living in transition/temporary situation or living in a facility where receives care; d, d, depressive symptoms was defined if the CES-D score was $\geqslant$10.

Table 4.2.2 Association between food insecurity and depressive symptoms among the Food Security & HIV-HCV Sub-study participants, Canada, 2012-2015

Methods	Food insecurity	Risk Ratio for Depressive Symptoms [c]
Crude (unadjusted)	Moderate	1.78 (1.57, 2.02)
	Severe	2.38 (2.14, 2.65)
Adjusted for baseline factors	Moderate	1.68 (1.47, 1.91)
	Severe	2.19 (1.95, 2.45)
Adjusted for baseline plus time-varying confounders [a]	Moderate	1.33 (1.20, 1.48)
	Severe	1.37 (1.25, 1.51)
Reweighted with SW [b]	Moderate	1.63 (1.44, 1.86)
	Severe	2.01 (1.79, 2.25)

a, past HIV clinical stage and past depression status were adjusted through regression method; b, the mean and standard deviation of stabilized weight used for this analysis is 1.002 and 0.147 with minimum/maximum values of 0.416/3.396; c, depressive symptoms was defined if the CES-D score was ≥10.

Table 4.2.3 Association between food insecurity and depressive symptoms with lagged exposure variable among the Food Security & HIV-HCV Sub-study participants, Canada, 2012-2015

Number of visits between exposure and outcome	Risk Ratio for Depression	Person-visit contribution
1	1.20 (1.02, 1.40)	1248
2	0.69 (0.54, 0.87)	640
3	0.94 (0.58, 1.52)	220

Figure 4.2.1 SW distribution across visits among the Food Security & HIV-HCV Sub-study participants, Canada, 2012-2015

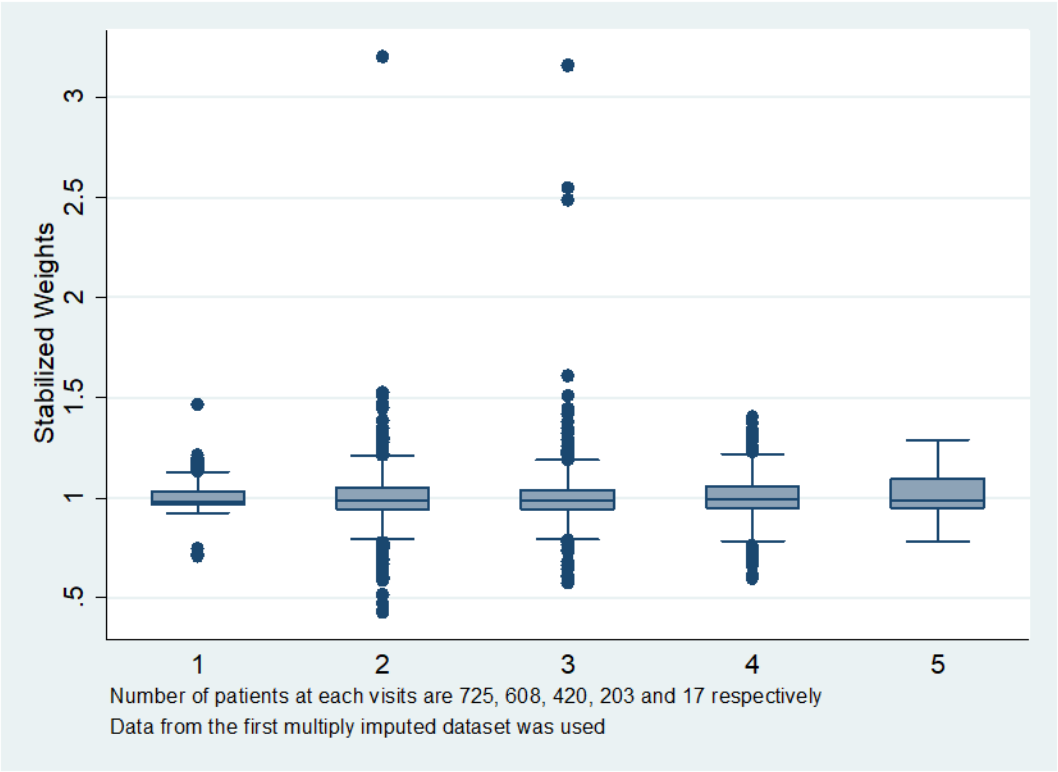
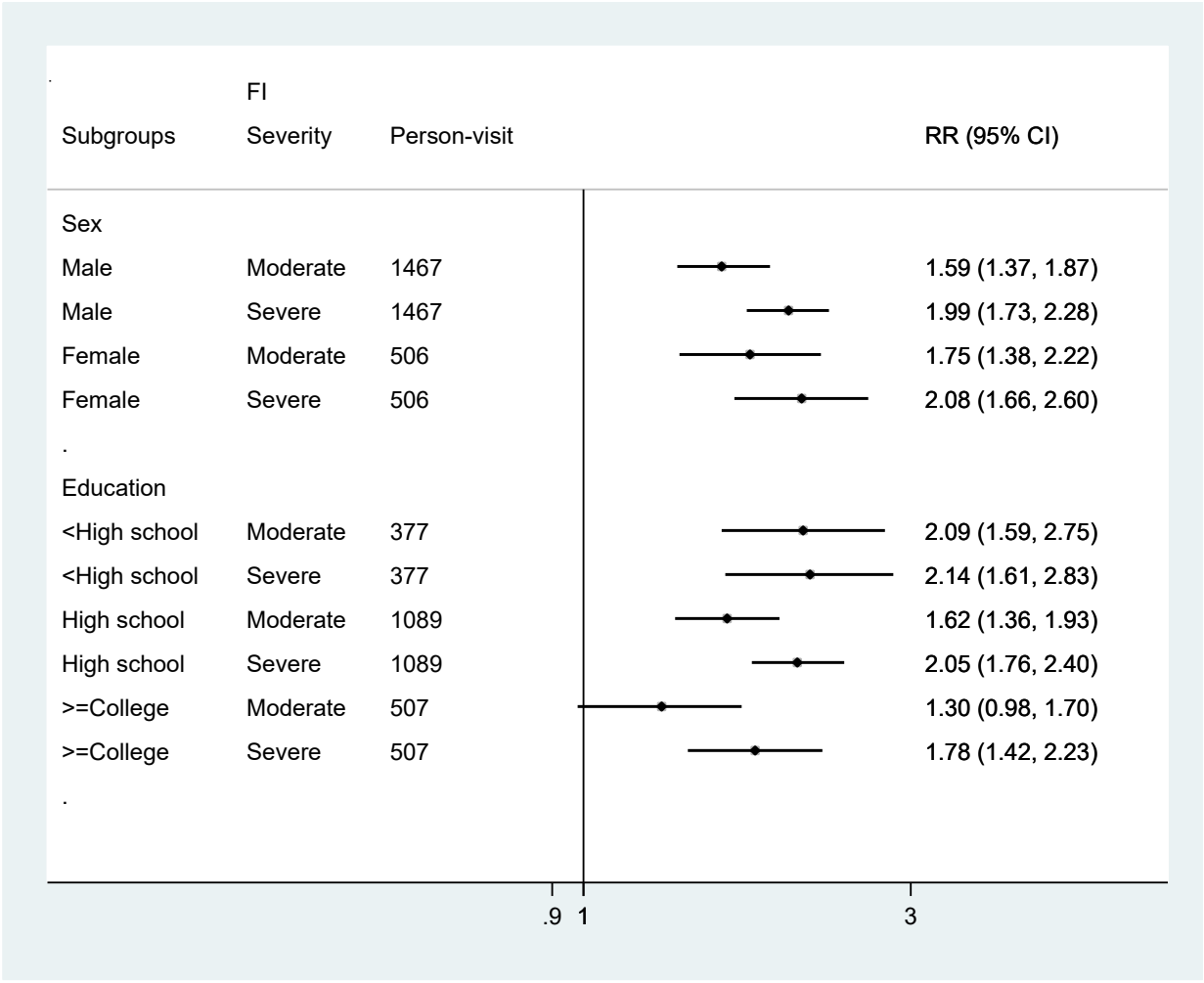


Figure 4.2.2 Subgroup analyses by sex and educational level of the association between food insecurity and depressive symptoms among the Food Security & HIV-HCV Sub-study participants, Canada, 2012-2015



Appendix 4.2.1 Calculation of Stabilized Inverse Probability Treatment Weights (IPTW)

IPTW was calculated using the following formula [124]

$$IPTW_{it}^{E} = \frac{\Pr[E_{it}|E_{it-1}, V_{io},\ \bar{C}_{jt} = 0]}{\Pr[E_{it}|E_{it-1}, V_{io},\ L_{it-1},\ \bar{C}_{jt} = 0]}$$

E_{it} represents participant i's FI status at visit t, V_{i0} includes baseline confounders, C_{jt} is censoring indicator. V_{i0} includes baseline confounders (sex, education level, race, marital status, age, IDU in the past 6 months and monthly income). L_{it-1} includes time-varying confounders.

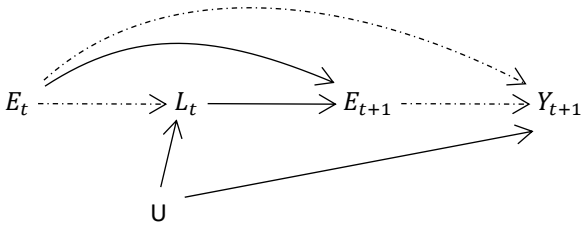
It should be noted that the above formula does not have cumulative multiplication mark as was suggested in previous literature [103,124]. Because our interest was to assess the effect of FI measured at visit k on depressive symptoms measured at visit k rather than to assess the effect of specific pattern of exposure (e.g. exposed at visits vs not exposed at all visits). Although exposure and outcome were measured at the same visit, the temporal order between them was assured through the time window in which they were measured. Specifically, the HFSSM measured participant's food experience over the past 6 months and the CES-D-10 measured depressive symptoms over the past week. Therefore, each visit can be conceived of a mini cohort study with an average follow up of 6 months and each person-visit was weighted with its own $IPTW_{it}^{E}$. The final result from weighted outcome model is a comparison of depressive symptoms when all study participants were exposed over the past 6 months vs. all study participants were not exposed over the past 6 months.

When t=0 (baseline visit), $E_{i,t-1}$= $E_{i,-1}$ and $L_{i,t\ -1}$= $L_{i,-1}$ and they represent exposure and time-varying confounders that should have occurred before baseline. In this analysis, $E_{i,-1}$ is FI status before baseline and $L_{i,-1}$ includes HIV clinical stage and depression status before baseline. Although it is possible to set their values to their corresponding baseline values, they are likely multifactorial and taking other factors into account may increase the chance of 'filling in' them correctly. Therefore, $E_{i,-1}$ and $L_{i,-1}$ were predicted by using sex, education level, race, marital status, IDU in the past 6 months, depression, HIV viral load, CD4 count.

Multinomial regression model was fitted to predict three level exposure probabilities both in the numerator and denominator in the weight model [103].

Appendix 4.2.2 Assumed Causal Structure between Variables

When analyzing the association between FI and depressive symptoms using data from a repeatedly measured cohort study, the depressive symptoms (outcome variable) will act as a time-varying confounder affected by prior exposure. Consider the following directed acyclic graph (DAG) (baseline confounders ignored) [150]

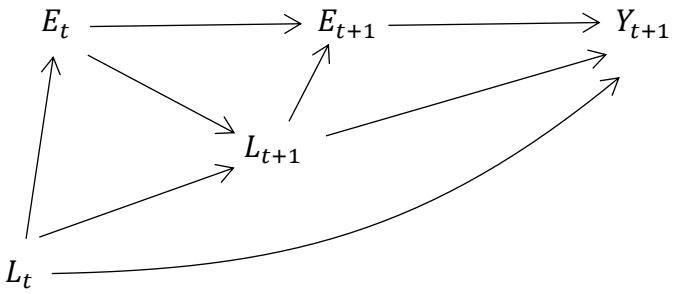


E_t is FI status measured at visit t and because of the nature of the questionnaire, it actually reflects participant's food insecurity experience over the past 6 months. L_t is depressive symptoms (and HIV clinical stage) measured at visit t. E_{t+1} is food insecurity status measured at visit t+1. Y_{t+1} is depressive symptoms measured at visit t+1. U includes unmeasured variables that may affect patient's depressive symptoms such as genetic predisposition for developing depression, intimate partner violence, etc. Dashed arrow represents the association between FI and depressive symptoms. It should be noted that L_t and Y_{t+1} contains the same variable (depressive symptoms), but they were measured at different study visits. For the association between E_{t+1} and Y_{t+1}, L_t is a confounder, because people who had depressive symptoms at visit t are also more likely to have depressive symptoms at visit t+1. It may also affect people's FI status (included in E_{t+1}) probably through lost of interest in pursuing employment opportunity or through worsened health status. In addition, studies among HIV infected people

indicated that food insecurity affects depressive symptoms (included in arrow from E_t to L_t) [42]. Therefore, outcome variables measured at prior visit may act as a time-varying confounder affected by prior exposure.

Appendix 4.2.3 Causal Mediation Analysis

In this analysis, we will use the following DAG to visualize relationships between variables.



In this DAG, E_t is exposure variable and it is FI status measured at visit t, E_{t+1} is mediator variable and it is FI status measured at visit t+1. Y_{t+1} is outcome variable and it is depression status measured at visit t+1. L_t is exposure-outcome confounders. L_{t+1} is mediator-outcome confounders. Since the exposure and mediator are the same variables (FI status) measured at different visits, L_t and L_{t+1} are also the same set of confounders measured at different visits. The complete list of variables included in them is,

L_t : sex (t), age (t), race (t), marital status (t), education level (t), income (t), IDU (t), HIV clinical stage (t-1) and depression (t-1).

L_{t+1} : sex (t+1), age (t+1), race (t+1), marital status (t+1), education level (t+1), income (t+1), IDU (t+1), HIV clinical stage (t) and depression (t).

The exposure-outcome confounder L_t also confounds the association between E_{t+1} and Y_{t+1}. In other words, L_t can also be a mediator-outcome confounder. However, once L_{t+1} (the mediator-outcome confounder of interest) is accounted for, L_t will not act as a mediator-

outcome confounder. Therefore, L_t was used for obtaining IPTW and L_{t+1} was used for obtaining stabilized inverse probability of mediator weights $IPMW_i^{E_{t+1}}$.

We assessed the controlled direct effect (CDE) [176] of E_t on Y_{t+1} by forcing all individuals to take the same value for E_{t+1} and pooled the CDE across visits. For the same reason as presented in Appendix 4.2.1, a visit specific stabilized inverse probability of mediator weights $IPMW_i^{E_{t+1}}$ were calculated without cumulative multiplication. Specifically, $IPMW_i^{E_{t+1}}$ was calculated using the following formula [176]

$$IPMW_{it+1}^{E_{t+1}} = \frac{\Pr[E_{it+1}|E_{it}]}{\Pr[E_{it+1}|E_{it},\ L_{it+1}]}$$

E_{it} is exposure variable (food insecurity measured at visit t), E_{it+1} is mediator variable (food insecurity measured at visit t+1), L_{it+1} includes mediator-outcome confounders. The $IPTW_{ij}^{E}$ was obtained with method detailed in Appendix 4.2.1 and the final stabilized weight (SW) was obtained as

$$SW_{ij} = IPTW_{ij}^{E} * IPMW_{ij}^{E_{t+1}}$$

After applying these weights to each person visit, mediator variable E_{t+1} was included in the outcome model to block the mediating effect of E_{t+1} in the association between E_t and Y_{t+1}

When the exposure was lagged by two study visits, an additional $\mathrm{IPMW}_{ij}^{E_{t+2}}$ was calculated using the following formula [176]

$$IPMW_{ij}^{E_{t+2}} = \frac{\Pr[E_{it+2}|E_{it+1}]}{\Pr[E_{it+2}|E_{it+1}, L_{it+2}]}$$

The final SW was obtained as

$$SW = IPTW_{ij}^{E} * IPMW_{ij}^{E_{t+1}} * IPMW_{ij}^{E_{t+2}}$$

After applying these weights to each person visit, mediator variables E_{t+1} and E_{t+2} were included in the outcome model to block the mediating effects of E_{t+1} and E_{t+2} in the association between E_t and Y_{t+2}

When the exposure was lagged by three study visits, another additional $\mathrm{IPMW}_{ij}^{E_{t+3}}$ was calculated using the following formula [176]

$$IPMW_{ij}^{E_{t+3}} = \frac{\Pr[E_{it+3}|E_{it+2}]}{\Pr[E_{it+3}|E_{it+2}, L_{it+3}]}$$

The final SW was obtained as

$$SW = IPTW_{ij}^{E} * IPMW_{ij}^{E_{t+1}} * IPMW_{ij}^{E_{t+2}} * IPMW_{ij}^{E_{t+3}}$$

After applying these weights to each person visit, mediator variables E_{t+1}, E_{t+2} and E_{t+3} were included in the outcome model to block the mediating effects of E_{t+1}, E_{t+2} and E_{t+3} in the association between E_t and Y_{t+3}.

Chapter 5: Assessing the Association between Mediator and Outcome

5.1 Preface to Manuscript 5

Food insecurity (FI) appears to be associated with depressive symptoms among HIV-HCV co-infected people. However, it is not clear if the presence of depressive symptoms is associated with poor HIV viral control and lower CD4 count among this population. Therefore, it is necessary to assess the latter association, to determine if the presence of depressive symptoms mediates the effect of FI on HIV viral load and CD4 count.

In this chapter, the associations between the presence of depressive symptoms, and both HIV viral load and CD4 count, among people co-infected with HIV-HCV was assessed. It is worth noting that the presence of depressive symptoms, HIV viral load and CD4 count were all measured at the same study visit. However, it is assumed that the temporal order between exposure and outcome is assured by the time window in which depressive symptoms was measured. Specifically, the short version of the Center for Epidemiologic Studies Depression Scale (CES-D) measured the presence of depressive symptoms during the past week, whereas CD4 count and HIV viral load measures were assumed to reflect their levels at the moment of sampling. Therefore, the time interval between exposure and outcome in this association is one week by design/default.

Depressive symptoms, HIV viral load, and CD4 count were all measured repeatedly over three years of follow up. Detectable HIV viral load and lower CD4 count at a previous visit predict HIV viral load and CD4 count at the current visit. Having detectable HIV viral load and lower CD4 count at a previous visit may influence severity of disease, contribute to people

feeling unwell or worried, and making them more likely to present with depressive symptoms at a subsequent visit. In other words, HIV viral load and CD4 count measured at a previous visit will confound the associations between presence of depressive symptoms, and both HIV viral load and CD4 count at a subsequent visit. Therefore, it is necessary to use appropriate analytical methods to account for this time-varying confounding. The inverse probability weighted marginal structural model was used to account for the confounding effect of past HIV viral load and past CD4 count.

5.2 Manuscript 5

Association between Depressive Symptoms, CD4 count and HIV Viral Suppression among HIV-HCV Co-Infected People

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Publication status: submitted to AIDS Care in September 2017

Abstract

Depressive symptoms are associated with poor HIV viral control and immune recovery among people living with HIV. However, no prior studies assessed this association exclusively among people co-infected with HIV-hepatitis C virus (HCV). While people living with HIV or HIV-HCV co-infection share many characteristics, co-infected people may experience additional vulnerabilities making them more susceptible to the effects of depressive symptoms on health outcomes. We assessed this association among people co-infected with HIV-HCV using data from the Food Security & HIV-HCV Sub-Study (FS Sub-Study) of the Canadian Co-Infection Cohort (CCC), a prospective cohort study covering 18 treatment sites across Canada. Depressive symptoms were measured using the short version of the Center for Epidemiologic Studies Depression Scale (CES-D). Potential time-varying confounders were accounted for using stabilized inverse probability weighting. A total of 725 participants were enrolled between 2012 and 2015. At baseline, 52% of participants reported depressive symptoms, 75% had undetectable HIV viral load, and median CD4 count was 466 (IQR 300-665). People experiencing depressive symptoms had 1.32 times (95% CI: 1.07, 1.63) the risk of having detectable HIV viral load, but had comparable CD4 count to people who did not experience depressive symptoms (fold change of CD4=0.96, 95% CI: 0.91, 1.03). Presence of depressive symptoms is a risk factor for incomplete short-term HIV viral suppression among people co-infected with HIV-HCV. While depressive symptoms did not confer an impact on CD4 count, depression screening and related counseling may improve HIV related health outcomes and reduce HIV transmission.

Key Words HIV-HCV co-infection; depressive symptoms; HIV viral load; CD4 count; inverse probability weighting.

Introduction

With the introduction and scaling up of combined antiretroviral treatment (cART) programs, HIV related mortality has steadily decreased around the world and the life expectancy of people living with HIV in high income countries is almost the same as for the general population [185]. Unfortunately, the presence of a disproportionate burden of other chronic conditions has coincided with the improvement in life span. For example, while the prevalence of depression is 4-5% among the general population [186], it is as high as 30% among people living with HIV [39,153,187]. Studies have also suggested that depression has a number of behavioral and clinical consequences such as lower quality of life [188], risky sexual behavior among men who have sex with men [44], poor adherence to cART regimens [189], and subsequently poor virologic control and immune recovery [190].

Despite evidence of the adverse health impacts of depression among people living with HIV, little is known about the HIV-related health effects of depression among HIV-hepatitis C virus (HCV) co-infected people. HCV infection is common among people living with HIV due to shared routes of transmission [19]. Approximately 15-30% of people living with HIV in North America are co-infected with HCV [191,192]. People co-infected with HIV-HCV are more likely to manifest depression than people with HIV only [193]; this is potentially related to the HCV activity in the central nervous system [160] and higher rates of injection drug use (IDU) [118,194]. Further, people co-infected with HIV-HCV tend to have significantly higher mortality compared to those with HIV only [148], possibly due to high rates of liver related deaths [195,196]. A recent cohort study also found that lack of HIV viral control and low CD4 count

were independent risk factors for advanced liver fibrosis [197], a prognostic factor of survival among people co-infected with HIV-HCV [198]. Therefore, understanding the association between depression, HIV viral suppression and CD4 count in co-infected individuals is an important step to improve survival in this population.

While findings from research on HIV mono-infected persons help inform our understanding of health outcomes among HIV-HCV co-infected persons, unique features of this population justify a focused enquiry. One such feature is high rates of IDU among co-infected patients. A recent study showed that 81% of HIV-HCV co-infected participants had a history of IDU and 34% reported actively injecting drugs over the past 6 months [194]. IDU is an independent risk factor for poor immune recovery and poor viral suppression [199-201]. Therefore, in the presence of additional risk factors, it is unclear to what extent the association between depressive symptoms and both HIV viral load and CD4 count holds among people co-infected with HIV-HCV. In this study, we assessed the association between depression, CD4 count and HIV viral load among people living with HIV-HCV co-infection in Canada.

Methods

Participants and Procedures

We used the Food Security & HIV-HCV Sub-Study (FS Sub-study) of the Canadian Co-Infection Cohort (CCC) [120,129], as depressive symptoms were measured only among the FS Sub-study participants. The detailed description of the CCC can be found elsewhere [120]. Briefly, the CCC study is a prospective cohort study involving 18 treatment sites across 6 Canadian provinces. In this cohort, patients co-infected with HIV-HCV aged>16 years were

recruited from clinic settings and followed every six months to collect sociodemographic, behavioral, clinical and laboratory information. The FS Sub-study was a cohort study initiated in 2012 within the CCC; its aim is to understand the occurrence of food insecurity, health related quality of life, and treatment outcomes among co-infected patients [129]. All CCC participants were invited to participate in the FS Sub-study; consented participants provided additional information on FI, depressive symptoms, health care utilization and medication adherence at each visit. As for the CCC study, information in the FS Sub-study was updated biannually until October 2015 [129]. Both the FS Sub-study and the CCC were approved by the research ethics boards of the participating institutions [120]; the secondary use of study data in this analysis was also approved by the McGill University Health Centre Research Ethics Board.

Measures

At each visit, self-administered questionnaires (interviewer assistance was also available) were used to collect data on variables, such as socio-demographic status, food insecurity, depressive symptoms, health care utilization, and housing situation [120,129]. HIV viral load and CD4 counts were measured at each study visit using standard procedures [120]. Depressive symptomatology was measured using the short version of the Center for Epidemiologic Studies Depression Scale (CES-D) [131], a 10-item questionnaire documenting the occurrence of depressive symptoms over the past week. Each item is assigned a score ranging from 0 to 3 based on responses from participants. The sum of the scores to all 10 items was used to determine the presence of depressive symptoms. Specifically, participants were considered to have depressive symptoms if the total sore was ≥10. The short version of the CES-D scale has good predictive accuracy (kappa=0.97) when compared to the original full scale [131], and has

been validated in different countries [168,169,202]. cART adherence was measured using items from the AIDS Clinical Trials Group (ACTG) Adherence Questionnaire [132,203]. In the current analysis, we used the question "If you have missed doses during past 4 days, how many days have you missed all doses?" to define adherence level [133]. Adherence was classified as perfect if there were zero reported missing days or imperfect otherwise. HIV viral load was classified as detectable if HIV viral load was ≥50 copies/mL and undetectable otherwise [130], whereas natural log transformed CD4 count was used as a continuous variable.

Statistical Analysis

Missing data were imputed using multiple imputation by chained equations [39], and 20 imputed data sets were created. Descriptive statistics of baseline variables were calculated and stratified by the presence of depressive symptoms. Continuous variables were compared using the Wilcoxon-Mann-Whitney test and categorical variables were compared using Chi-square test. To obtain an exposure effect with a population average ("marginal") interpretation, stabilized inverse probability weights (SWs) were applied to account for potential time-varying confounders (Appendix 5.2.1) [103,124]. Sex, race, marital status, education level and injection drug use (IDU) were used as baseline confounders and FI, past HIV viral load and past CD4 count were used as time-varying confounders. Since the inclusion of past HIV viral load and past CD4 count will account for the potential confounding effects of cART treatment status and medication adherence, these two variables were not used as confounders (Appendix 5.2.2).

A weighted linear regression model was used for the association between depressive symptoms and log transformed CD4 count and a weighted Poisson regression model with log

link was used for the association between depressive symptoms and detectable HIV viral load (≥ 50 copies/mL) [137]. Within subject clustering potentially induced by repeated measurement and weighting may increase type I error and therefore a robust sandwich variance estimator was specified to obtain conservative confidence intervals. The final point estimate and variances were combined across 20 imputed datasets using Rubin's rules [139].

To assess the dose response relationship in terms of duration of depression, we redefined exposure as depression that occurred at two or more consecutive study visits. In this redefinition, if a person was depressed intermittently across visits, then his/her exposed person-visits were discarded due to the non-consecutive exposure pattern and only unexposed person-visits were retained. The same potential confounders as in the main analysis were used to obtain SW.

In the above analyses, exposure and outcome were measured at the same study visit and we assumed the temporal order between exposure and outcome was assured by the one-week time window for the measurement of depressive symptoms. In other words, the CES-D questionnaire was administered at the same visit when blood samples were taken for CD4 count and HIV viral load. Because of this temporal proximity (i.e. the CES-D questionnaire asks for participants' depressive symptoms during the previous week and CD4 count and HIV viral load measures reflect the moment of sampling), we conducted additional analyses by lagging exposure by one visit. We hypothesized that a one-week interval may be too short for the exposure to exert an effect on the outcome and a lagging approach could help capture a possible effect.

In addition, because successfully treated HIV-positive patients may experience isolated viral blips (transiently detectable low level viremia, typically HIV RNA <400 copies/mL), which are not predictive of virologic failure [204,205], we conducted sensitivity analyses using a more conservative threshold (≥400copies/mL) for detectable HIV viral load. We used the same statistical methods elaborated above to assess the robustness of the results.

Results

A total of 725 HIV-HCV co-infected participants were enrolled in this study. Over the three years of follow up, the number of participants who completed two or more, three or more and four or more visits were 608, 420 and 203 respectively; the number of participants identified as having depressive symptoms at two or more, three or more and four or more consecutive visits were 258, 134 and 55 respectively. Compared to participants who did not report depressive symptoms, participants with depressive symptoms were more likely to have a lower monthly income, food insecurity and to have injected drugs over the past six months. Also, participants with depressive symptoms were less likely to have perfect cART adherence and more likely to have detectable HIV viral load. There was no difference observed in terms of age, sex, race, education, marital status and HIV clinical stage (Table 5.2.1).

The SW weighted model without lagged exposures indicated that participants with depressive symptoms had 1.32 (95% CI 1.07-1.63) times the risk of having detectable HIV viral load (≥50 copies/mL) compared to participants without depressive symptoms. However, participants with depressive symptoms had comparable CD4 counts to those without depressive symptoms (fold change of CD4=0.96, 95% CI 0.91, 1.03). When exposure was lagged

by one visit, depressive symptoms were not associated with either outcome (Table 5.2.2). The distribution of SW used for the non-lagged analysis is presented with a visit specific boxplot in Figure 5.2.1.

When the exposure was defined as having depressive symptoms at two or more consecutive visits (Table 5.2.3), the strengths of the associations for both HIV viral load and CD4 count did not differ from those presented in Table 5.2.2.

Sensitivity analyses redefining detectable HIV viral load with a higher threshold (≥400copies/mL) yielded stronger associations. Participants reporting depressive symptoms had a significantly higher risk of having detectable HIV viral load compared to those who did not have depressive symptoms (RR=1.57, 95% CI 1.15-2.15). When exposure was lagged by one visit, the association became borderline non-significant (RR=1.42, 95% CI 0.94, 2.16).

Discussion

This is the first cohort study to assess the association between depressive symptoms, HIV viral load and CD4 count among HIV-HCV co-infected patients. Our results indicate that the presence of depressive symptoms is a significant risk factor for incomplete HIV viral suppression. However, this association disappeared when we allowed 6 months for the interval between exposure and outcome. Previous studies suggested that depressed patients are less likely to adhere to cART [189] and poor adherence may cause viral rebound [206], indicating that at least part of the effect of depression on incomplete HIV viral suppression is mediated through cART adherence. As a clinical cohort, it is possible that participants presenting with depressive symptoms are identified as at-risk for poor adherence, triggering preventive adherence related

interventions by on-site health providers (e.g. closer follow up). This may have resulted in better control of HIV at the subsequent visit regardless of the experience of depressive symptoms at the previous visit. Another possible explanation for this short-term effect is likely the detection capacity of the CESD scale. Since it is designed to screen for depression among the general population, it has higher sensitivity with lower specificity. Therefore, it may have captured transient and reactive depressive symptoms rather than chronic clinical depression. These transient symptoms may quickly dissipate with improvement on related triggers such as transient financial difficulties. Lastly, it is unclear if participants who reported depressive symptoms received any form of counseling. Receiving such counseling may have improved depressive symptoms before the next visit.

Given the overall low level of HIV viral load among this study population, our definition of detectable HIV viral load in this study (≥50 copies/mL) likely captured viral blips which is not necessarily an indication of active viral replication [204]. Therefore, we redefined detectable HIV viral load with a higher threshold (≥400 copies/mL) and repeated the same analysis. The result of the sensitivity analysis showed an even stronger association between presence of depressive symptoms and detectable HIV viral load. In addition, we empirically evaluated the occurrence of virologic rebound after having virologic suppression as per the US Department of Health and Human Services [205]. Interestingly, the proportion of person-visits with virologic rebound among those who reported depressive symptoms is 1.7 times the proportion of those with a virologic rebound without reporting depressive symptoms (3.9% vs. 2.3%). Together, these findings suggest the presence of depressive symptoms as a possible early precursor to

HIV viral replication which could undermine efforts to improve individual health and limit ongoing transmission of HIV.

In both the lagged and unlagged analyses, there is little evidence of association between depressive symptoms and CD4 count even though depression was negatively associated with HIV viral control in the unlagged analysis. Previous epidemiologic studies among people living with HIV with unknown proportion of HCV co-infection have shown that there was a strong association between depression and lower CD4 count [190,207]. Based on the dynamics between HIV viral load and CD4 count, one would expect patients with higher HIV viral load to eventually experience lower CD4 counts [14]. Although it is difficult to pinpoint the exact reasons for our discrepant results, characteristics of the study design and study participants may provide some insights. First, in the unlagged analysis, the exposure-outcome interval was one week by design and may be too short to observe a significant CD4 cell decline given the estimated half-life of HIV infected CD4 cells of 14-28 days [208]. Second, although we found a significant association between depressive symptoms and HIV viral suppression in the unlagged analysis, the overall viral load in this sample was low. Only 10% of the patients with depressive symptoms had HIV viral load above 2500 copies/mL and 10% of the patients without depressive symptoms had an HIV viral load above 200 copies/mL at baseline. This suggests that HIV mediated CD4 cell destruction was very limited and possibly caused similar degrees of CD4 cell decrease regardless of the presence of depressive symptoms. Indeed, in one study that demonstrated an association between depression and lower CD4 count, participants had HIV viral loads of 4.38 (in log10 scale) on average at study entry [190] while our study participants had HIV viral loads of 1.88 (in log10 scale) on average at study entry.

To capture persistent depressive symptoms rather than transient and reactive ones, exposure was redefined as having depressive symptoms at two or more consecutive study visits and the association with HIV viral load and CD4 count was reassessed, using the same method as for the main analyses. However, this redefinition did not alter the original associations meaningfully (Table 5.2.3). While this could be an indication that there is no dose response relationship in terms of the duration of depressive symptoms, the overall high sensitivity and lower specificity of the screening tools must be considered. Our study is not powered to assess a dose response relationship in terms of severity of depressive symptoms although there appears to be a dose response trend in point estimates for detectable HIV viral load (data not shown). Future studies may consider assessing a dose response relationship in terms of severity of depressive symptoms with a larger sample size because it could help prioritize certain groups for intervention.

This study has several strengths. First, the prospective nature of our study enabled us to assess the exposure-outcome association by assuring a clear temporal order between exposure, outcome and potential confounders. Second, because regular regression adjustment methods may not adequately adjust for time-varying confounders, we used an epidemiologic method specifically designed to tackle this problem [103,124]. Third, the study population is comprised exclusively of people co-infected with HIV-HCV. Since poor HIV viral control and immune recovery increase the risk of severe liver disease among people co-infected with HIV-HCV [209-211], identifying potential factors associated with poor HIV viral control may provide clues for interventions by health professionals. However, there are also several limitations. First, the relatively short interval between exposure and outcome may have prevented us from fully

understanding the association between depressive symptoms and immune status measured via CD4 count. Second, while our study is a prospective cohort study that measured many potential confounders, as in any other observational study, our exposure was not 'allocated randomly'. Therefore, we acknowledge the possibility of residual confounding. Third, although depressive symptoms were measured with a validated tool [131], screening positive does not necessarily mean the subject has a clinical depression status (screening positive means that an individual has a higher probability of having depression and needs further clinical evaluation). Therefore, in some of those who screened positive, depressive symptoms were likely transient. Fourth, even though ≥50 copies/mL was used as the threshold for detectable HIV viral load [130], this threshold may decrease with advances in testing technology. Therefore, we empirically assessed the number of person visits that would have been classified as detectable under a new threshold of ≥40 copies/mL and identified an additional 40 person-visits, or 2% of total person-visits, as detectable. This indicates that our results were relatively robust even if a lower threshold for HIV viral load was used.

In conclusion, we found that depressive symptoms are associated with detectable HIV viral load among HIV-HCV co-infected people. In contrast, there was no impact on CD4 counts. Therefore, screening for depressive symptomatology may be useful in identifying persons at-risk for short-term viral replication. Interventions targeting the causes of these symptoms could help limit secondary HIV transmission.

Table 5.2.1 Baseline sociodemographic and clinical characteristics of participants in the Food Security & HIV-HCV Sub-study, Canada, 2012-2015

Characteristics	Total (N=725) [a]	Absence of depressive symptoms (n=340)	Presence of depressive symptoms (n=377)	P
Median age in years (IQR) [b]	49 (43, 54)	50 (43, 55)	48 (43, 53)	0.12
Male	528 (73%)	251 (74%)	272 (72%)	0.52
Caucasian	542 (75%)	241 (71%)	297 (79%)	0.11
Married or common law	133 (18%)	71 (21%)	61 (16%)	0.25
College or greater education	190 (26%)	94 (28%)	94 (25%)	0.4
Monthly income below 1000 CAD [c]	308 (42%)	120 (35%)	185 (49%)	<0.001
Any food insecurity [d]	416 (57%)	140 (41%)	271 (72%)	<0.001
IDU [e] in the past 6 months	238 (33%)	84 (25%)	151 (40%)	<0.001
Clinical Stage III	43 (6%)	20 (6%)	23 (6%)	0.9
Median time since HIV infection (IQR), years	14.1 (8.3, 18.4)	14.3 (8.1, 19.2)	13.4 (8.6, 17.4)	0.27
On cART	662 (91%)	315 (93%)	340 (90%)	0.24
Perfect adherence [f]	531 (80%)	290 (85%)	262 (70%)	<0.001
Median CD4 count (IQR), cell/ul	466 (300, 665)	481 (301, 677)	450 (300, 645)	0.32
Undetectable HIV RNA (<50 copies/mL)	547 (75%)	368 (79%)	274 (73%)	0.004

a, 8 participants had missing values for depressive symptoms and therefore sum of exposed and unexposed individuals does not equal to total number of participants; b, IQR, Interquartile Range; c, CAD, Canadian Dollars; d,

any food insecurity includes moderate food insecurity and severe food insecurity; e, IDU, injection drug use; f, sample restricted to those who were taking cART.

Table 5.2.2 Association between depressive symptoms and both HIV viral load and CD4 count among participants in the Food Security & HIV-HCV Sub-study, Canada, 2012-2015

Exposure Lagged	Relative Risk for detectable HIV viral load [a]	Fold change in CD4 count [b]	Person-visit contribution
No	1.32 (1.07, 1.63)	0.96 (0.91, 1.03)	1973
Yes	1.13 (0.86, 1.48)	0.96 (0.89, 1.04)	1248

a, the threshold is defined as ≥50 copies/mL for detectable HIV; b, fold change describes the average change of CD4 count when comparing exposure groups. For example, the point estimate of this result indicates that the average CD4 count among exposed group is equivalent to 96% of those unexposed. In other words, exposed group has 4% fewer CD4 counts than unexposed group.

Table 5.2.3 Association between depressive symptoms and both HIV viral load and CD4 count when exposure was defined as two or more consecutive depressive symptoms among participants in the Food Security & HIV-HCV Sub-Study, Canada, 2012-2015

Exposure Lagged	Relative Risk for detectable HIV viral load [a]	Fold change in CD4 count [b]	Person-visit contribution
No	1.34 (1.06, 1.68)	0.95 (0.89, 1.01)	1703
Yes	1.19 (0.89, 1.59)	0.95 (0.88, 1.03)	1117

a, the threshold is defined as ≥50 copies/mL for detectable HIV; b, fold change describes the average change of CD4 count when comparing exposure groups. For example, the point estimate of this result indicates that the average CD4 count among exposed group is equivalent to 96% of those unexposed. In other words, exposed group has 4% fewer CD4 counts than unexposed group.

Appendix 5.2.1 Calculation of Stabilized Weights (SWs)

SW was calculated using the following formula [103,124]

$$SW_{ik}^{E} = \frac{\Pr[E_{ik}|E_{ik-1},V_{io},\bar{C}_{jk}=0]}{\Pr[E_{ik}|E_{ik-1},L_{ik-1},\bar{C}_{jk}=0]}$$

E_{ik} represents participant i's depressive symptoms at visit k and it measures patient's depressive symptoms over the past week. V_{i0} includes baseline confounders (sex, race, marital status, education level, IDU). C_{jk} is censoring indicator. L_{ik-1} includes all the variables included in V_{i0} plus past HIV viral load and past CD4 count.

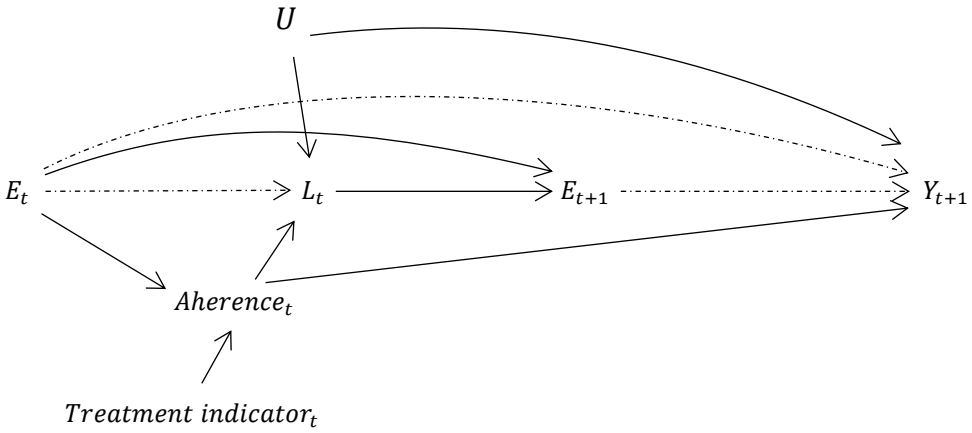
It should be noted that the above formula does not have cumulative multiplication mark as was suggested in previous literature [103,124]. Because our interest was to assess the effect of depressive symptoms measured at visit k (depressive symptoms over the past week) on HIV viral load and CD4 count measured at visit k rather than to assess the effect of specific pattern of exposure (e.g. exposed all the time vs not exposed all the time). Therefore, each person-visit was weighted with its own SW_{ik}^{E}. Subsequently, the result from the weighted outcome model is a comparison of HIV viral load and CD4 count when all study participants were exposed over the past week vs. all study participants were not exposed over the past week.

In the calculation of the exposure probabilities at baseline visit, we need to use unmeasured values, $E_{i,-1}$ and $L_{i,-1}$. Since cohort data collection does not correspond to any conceptual time zero, it is impossible to simply set $E_{i,-1}$ and $L_{i,-1}$ to any specific values. In other words, people could be depressed or not depressed at potential visit that could have occurred before baseline visit. One possible way to address this issue is to set both $E_{i,-1}$ and

$L_{i,-1}$ to their corresponding baseline values. For example, patients with depressive symptoms at baseline were also more likely to be depressed at potential visit that could have occurred before baseline. However, depressive symptoms (included in $E_{i,-1}$), HIV viral load (included in $L_{i,-1}$) and CD4 count (included in $L_{i,-1}$) are likely multifactorial and taking other factors into account may increase the chance of 'filling in' their values correctly. Therefore, $E_{i,-1}$ and $L_{i,-1}$ were predicted by arranging data set in long format and using food insecurity, sex, education level, marital status, IDU, depression, HIV viral load, CD4 count. By arranging the data set in long format, the strength of association between $E_{i,k}$ and $E_{i,k+1}$ within cells defined by $V_{i,0}$ and $L_{i,k}$ can be utilized to 'fill in' the most probable values of $E_{i,-1}$; and the strength of association between $L_{i,k}$ and $L_{i,k+1}$ within cells defined by $V_{i,0}$ and $E_{i,k}$ can also be utilized to 'fill in' the most probable values of $L_{i,-1}$.

Appendix 5.2.2 Assumed Causal Structure between Variables

When analyzing the association between depressive symptoms and both HIV viral load and CD4 count, using data from a repeatedly measured cohort study, the HIV viral load and CD4 count (outcome variables) measured at prior visit will act as time-varying confounders for the subsequent association. Consider the following directed acyclic graph (DAG) [150]



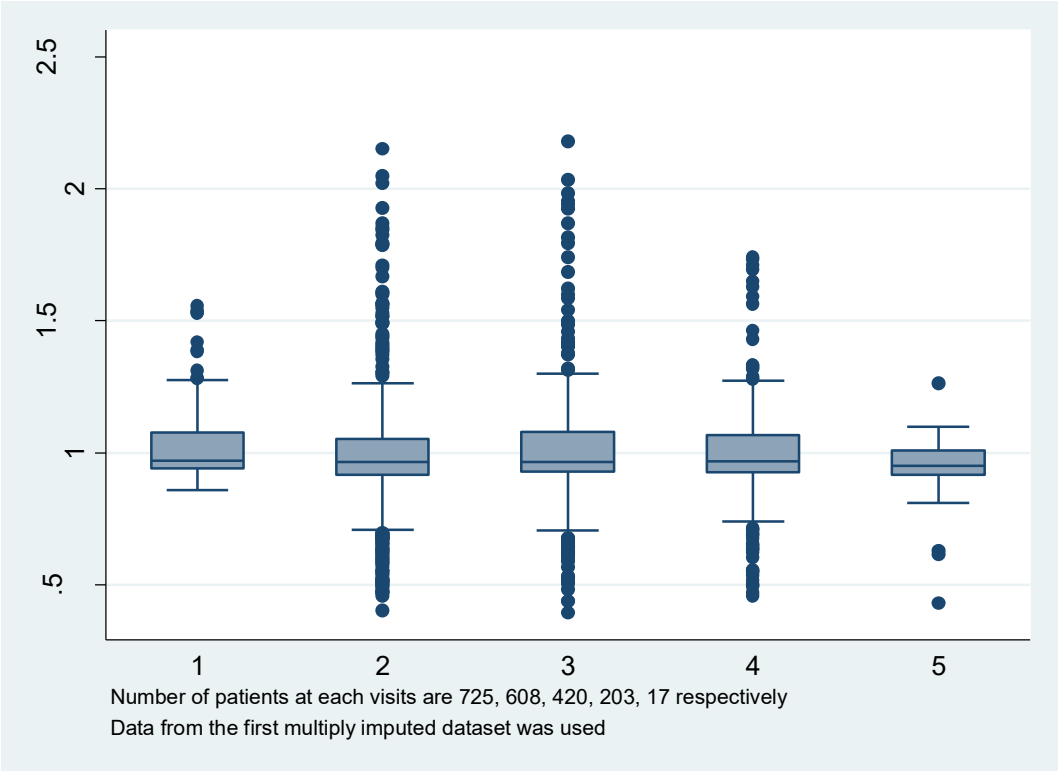
E_t is depressive symptoms measured at visit t. $Adherence_t$ is adherence level measured at visit t. $Treatment\ indicator_t$ is a variable that indicates whether a patient was on cART or not at visit t. L_t is HIV viral load and CD4 count measured at visit t and Y_{t+1} is HIV viral load and CD4 count measured at visit t+1. U includes unmeasured variables that affect patient's immune status such as genetic factors, exercise level, health awareness, etc. Because of the measurement window of the CES-D scale [131], which measures participant's depressive symptoms over the past week, we assumed that E_t precedes L_t.

Dashed arrow represents the association between depressive symptoms and both HIV viral load and CD4 count. People with low CD4 count and high HIV viral load may be sicker, more stressed, and therefore more likely to present with depressive symptoms at subsequent visit (represented by an arrow from L_t to E_{t+1}). Weak immune status at visit t is also a predictor of immune status at visit t+1. Therefore, HIV viral load and CD4 count measured at visit t (represented with L_t) can be a time-varying confounder for the association between depressive symptoms and both HIV viral load and CD4 count measured at visit t+1 (the association between E_{t+1} and Y_{t+1}).

$Adherence_t$ is a predictor of HIV viral load and CD4 count (the arrow from $Adherence_t$ to L_t and from $Adherence_t$ to Y_{t+1}). It also affects E_{t+1} through worsened health status (represented with arrows $Adherence_t \rightarrow L_t \rightarrow E_{t+1}$). $Adherence_t$ is likely affected by depressive symptoms (E_t). Therefore, $Adherence_t$ is a time-varying confounder affected by prior exposure. Only patients who started cART can be evaluated on adherence. Therefore, $Treatment\ Indivator_t$ is upstream variable relative to $Adherence_t$.

Since the confounding effect of $Treatment\ indicator_t$ will act through $Adherence_t$ and the confounding effect of $Adherence_t$ act through L_t (HIV viral load and CD4 count), L_t is more downstream confounder relative to both $Treatment\ indicator_t$ and $Adherence_t$. Accounting for downstream confounder is equivalent to accounting for upstream confounders. Therefore, inclusion of L_t in the weight SW model should be enough to control for confounding effect of $Treatment\ indicator_t$ and $Adherence_t$.

Figure 5.2.1 Stabilized weight distribution across study visits among the Food Security & HIV-HCV Sub-study participants, Canada, 2012-2015



Chapter 6: Stochastic Mediation Analysis with an Outcome Redistribution Approach

6.1 Preface to Manuscript 6

In chapter 3, overall associations between food insecurity (FI) and both HIV viral load and CD4 count were assessed. The results in chapters 4 and 5 indicated that the presence of depressive symptoms lies on the pathway between FI and HIV viral load, but not with respect to CD4 count. Therefore, it is possible that a certain amount of FI effect on HIV viral load could be blocked by an effective intervention on depressive symptoms, whereas the FI effect on CD4 count could remain unchanged.

The main goal of mediation analyses is to provide alternative targets for public health intervention. In addition, it is possible to find more effective targets to block detrimental exposure effects on an outcome by conducting mediation analyses on more than one mediator. In this chapter, mediation analyses were undertaken for two potential mediators in the associations between FI and both HIV viral load and CD4 count. The first analysis used depressive symptoms as the mediator, while the second used combined antiretroviral treatment (cART) adherence as the mediator. From public health and clinical intervention perspectives, the mediator that completely blocks the exposure effect is regarded as an effective mediator compared to the one that only partially blocks the exposure effect.

Causal mediation analysis proposes to use inverse probability weighted marginal structural model to quantify the controlled direct effect (CDE) when there are mediator-

outcome confounders. However, this approach implicitly assumes that the intervention on the mediator is 100% effective, which is almost impossible in real-world intervention settings. The stochastic mediation contrast method, on the other hand, mimics different intervention effectiveness levels by altering only a fraction of the observations' mediator values. However, this method may have limitations in cohort studies involving repeated measures due to the potential presence of time-varying mediator-outcome confounders.

In this chapter, an alternative method is explored by taking advantage of both the marginal structural model and the stochastic mediation contrast method. Weighting with inverse probability of exposure and mediator allows this method to be used in the presence of time-varying mediator-outcome confounders and the stochastic approach allows this method to mimic the real-world intervention scenario.

6.2 Manuscript 6

Mechanism for the Negative Impact of Food Insecurity on HIV Viral Load and CD4 Count among HIV-HCV co-infected People: A Stochastic Mediation Analysis with an Outcome Redistribution Approach

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Publication status: Submitted to Epidemiology in September 2017

Abstract

Background: Although prior studies identified potential pathways through which food insecurity (FI) may exert harmful effect on HIV viral control and CD4 count, no study quantified the strength of pathways among people co-infected with HIV-HCV. In this study, two potential pathways, depressive symptoms and combined antiretroviral treatment (cART) adherence, were assessed exclusively among people co-infected with HIV-HCV.

Methods: Data from 725 people co-infected with HIV-HCV recruited across 6 Canadian provinces over 3 years of follow-up was analyzed. The controlled direct effects (CDE) of severe FI on HIV viral control and CD4 count were assessed using stochastic mediation analysis with outcome redistribution approach.

Results: The CDE of severe FI on HIV viral load decreased with the increased effectiveness of intervention on mediators. However, considerable amount of FI effect remained even with hypothetical 100% effective interventions on depressive symptoms (CDE=1.33, 95% CI: 1.02, 1.74) and on cART adherence (CDE=1.35, 95% CI: 0.97, 1.87). No mediating role of depressive symptoms and cART adherence were observed in the association between severe FI and CD4 count.

Conclusion: Intervention on cART adherence and/or depressive symptoms was not sufficient to block the detrimental effect of severe FI on HIV viral load and CD4 count among people co-infected with HIV-HCV.

Key words: HIV-HCV co-infection, food insecurity, HIV viral load and CD4 count, mediation analysis.

Introduction

Food insecurity (FI), defined as a limited or uncertain availability of nutritionally adequate and safe foods, or limited or uncertain ability to acquire acceptable foods in socially acceptable ways [1], is common among HIV infected people. Previous studies conducted in US and Canada consistently estimated that roughly half of their respective study samples were experiencing various levels of FI [24,25,42]. FI is associated with poor HIV virologic control and immune reconstitution [26,75,76]. Recent meta analyses also showed that people experiencing FI had 32% more odds to have lower CD4 count [141] and 29% less odds to achieve HIV viral suppression [212]. Although the exact mechanism for this association is unknown, studies indicated that FI may lead to depressive symptoms [42,157]; depressive symptoms are associated with poor combined antiretroviral treatment (cART) adherence [47,213], which in turn is a direct cause for subsequent viral rebound [143,214]. Therefore, the plausible mechanism could be through depressive symptoms and poor cART adherence. However, other pathways such as nutritional compromise are equally plausible [122]. Experiencing FI may introduce different coping behaviors such as reduced consumption of food and relying on less expensive and less nutritious foods, leading to nutritional deficiencies over the long term [60]. Adequate nutrition is essential in preserving numerical and functional efficiencies of cell immunity [81] and increasing its effectiveness against HIV. However, the magnitude and strength of the pathways through which FI is associated with poor HIV viral control and lower CD4 count has not been well described.

Identification and quantification of exposure effect through different pathways may be of policy interest, as it may provide potential targets for public health intervention that are either cost effective or amenable to intervention or simply better accepted by the target population than intervention on exposure itself. Conventionally, a regression adjustment for a mediator variable is used to find direct exposure effect. Specifically, the mediator variable is included in the regression model and the coefficient corresponding to the exposure variable is regarded as "direct exposure effect". However, it was shown that this conventional approach may not provide unbiased estimation of direct effect when there are mediator-outcome confounders [181,215-217]. VanderWeele TJ [176] proposed the marginal structural model to quantify the controlled direct effect (CDE). In this method, inverse probabilities of exposure and mediator are calculated based on exposure-outcome and mediator-outcome confounders and the mediator will be included in the weighted regression model. Although this method allows for the correct estimation of CDE, it quantifies the exposure effect that would remain if the mediator were set to a single value, uniformly in the population (i.e. that intervening on the mediator is 100% effective), which is not likely to be realized in practice.

To address this issue, Naimi and colleagues [218] suggested stochastic mediation contrast, a method where only a portion of the target population may be affected by intervention on the mediator. In this method, a regular regression is fitted to quantify the mediator contribution to the outcome distribution and the outcome is transformed by factoring the mediator contribution out either through subtraction or division [218]. In point exposure, mediator and outcome settings, as was the case in that paper, the mediator contribution can be correctly identified using regular regression method. In cohorts involving repeated measures,

however, it is possible to have time-varying mediator-outcome confounders affected by previous mediator; direct conditioning on mediator-outcome confounders may not be sufficient to quantify the mediator contribution. In this paper, we explored an alternative approach that is built upon the marginal structural model [176] and the stochastic mediation contrast method [218], and redistributes the outcome by respecting its original distributional property. Using this method, we sought to estimate the mediating role of depressive symptoms and cART adherence in the association between FI and both HIV viral load and CD4 count among HIV-HCV co-infected people in Canada.

Methods

Study Population and Variable Measurement

The study population is comprised of participants in the Food Security & HIV-HCV Sub-Study (FS Sub-study) of the Canadian Co-Infection Cohort (CCC) [120,219]. The recruitment process, measurement of variables, and follow-up procedure of the CCC has been previously described [120]. Briefly, the CCC study is a prospective cohort study that was initiated in 2003 and recruited HIV-HCV co-infected patients aged>16. The CCC includes 18 treatment sites across 6 provinces in Canada and updates sociodemographic, behavioral, clinical and laboratory information every six months. The FS Sub-study was conducted (2012-2015) within the CCC [219]. All CCC participants were invited to participate in the FS Sub-study and participants provided additional information on FI, depression, health care utilization, and cART adherence at each visit. As in the CCC, information in the FS Sub-study was collected biannually using questionnaires [219]. Both the FS Sub-study and the CCC were approved by the research ethics

boards of the participating institutions [120], and the secondary use of data for this analysis was approved by the McGill University Health Centre Research Ethics Board.

The exposure variable (FI) was measured using the 10-item adult scale of Health Canada's Household Food Security Survey Module (HFSSM) [220]. In accordance with the Canadian Community Health Survey (CCHS) categorization [220], it was classified into three categories: food security (0 or 1 affirmative answer on the HFSSM), moderate food insecurity (2-5 affirmative answers) and severe food insecurity (≥6 affirmative answers). The first mediator variable (depressive symptoms) was measured using the short version of the Center for Epidemiologic Studies Depression Scale (CES-D) [131], a 10-item questionnaire assessing occurrence of depressive symptoms over the past week (CES-D-10). This short version of the CES-D scale was previously validated in various countries and study populations [168,169,202]. Each item in the CES-D-10 is scored from 0 to 3 and the total score ranges from 0 to 30. Participants were considered to have depressive symptoms if the total score was $\geqslant$10 [131,168]. The second mediator variable (cART adherence) was measured using the AIDS Clinical Trials Group (ACTG) Adherence Questionnaire [132,203]. In this analysis, we used the question "If you have missed doses during past 4 days, how many days have you missed all doses?" to define adherence level [133]. Adherence was classified as perfect if there was zero reported missing day and imperfect otherwise. Outcome variables (HIV viral load and CD4 count) were measured using standard laboratory procedures biannually [120]. HIV viral load was classified as detectable ($\geqslant$50 copies/mL) and undetectable [130], whereas natural log transformed CD4 counts were used as a continuous variable.

Statistical Methods

To review and illustrate related statistical methods, we will use the directed acyclic graph (DAG) in Figure 6.2.1 to visualize assumed causal relationships between variables [150,221].

The direct effect of A on Y that is not mediated through M includes $A - Y$ and $A - L - Y$. In the conventional method, direct effect is assessed using the following regression equation

$$E(Y|C, M) = \beta_0 + \beta_1 A + \beta_2 M + \beta_3 C \qquad (Model\ 1)$$

Because of the existence of mediator-outcome confounder L, conditioning on mediator M may introduce collider stratification bias [123] and therefore, β_1 in model 1 may not correspond to the direct effect of A. In the marginal structural model approach [176], inverse probabilities of exposure and mediator weights (IPTW and IPMW hereafter) are calculated as

$$IPTW = \frac{\Pr[A = a]}{\Pr[A = a|C = c]}$$

and

$$IPMW = \frac{\Pr[M = m|A = a]}{\Pr[M = m|A = a, L = l, C = c]}$$

after each observation is weighted with corresponding stabilized weights $SW = IPTW * IPMW$, a marginal structural model will be fitted to obtain direct effect

$$E(Y_{am}) = \alpha_0 + \alpha_1 a + \alpha_2 m + \alpha_3 am \qquad (Model\ 2)$$

Y_{am} denotes a subject's counterfactual value for Y if A were set to a and M were set to m [176]. Weighting with SW will create a pseudo population in which M is independent from C and L

[103], and prevent M being a collider. Coefficient $\alpha_1 + \alpha_3$ in marginal structural model 2, therefore, corresponds to CDE [176]. CDE calculated from the marginal structural model 2 is the exposure effect when mediator M for all study subjects is set to m. In other words, CDE is the magnitude of exposure effect when intervention on mediator is 100% effective. Although CDE calculated from this method is an important theoretical quantity, it hardly reflects a real-world scenario where intervention is almost always suboptimal.

The stochastic mediation contrast, on the other hand, is an approach where only a fraction of study subjects' mediator variable is altered to represent a suboptimal intervention scenario [218]. In this method, a standard regression model will be fitted as first step

$$E(Y|A, M, C, L) = \beta_0 + \beta_a A + \beta_m M + \beta_{am} AM + \beta_c C + \beta_l L \qquad (Model\ 3)$$

In step two, a portion of subjects will be selected from people whose mediator is not at reference level with the selection probability,

$$\Pr(R = 1|A, C) = \{1 + \exp(-(\delta_0 + \delta_1 A^* + \delta_2 C^*))\}^{-1} \quad if\ M \neq reference\ level$$

where δ_n are user defined coefficients to reflect plausible assumption about the effectiveness of intervention on mediator. A^* and C^* are selected to reflect targeted subgroup for mediator intervention (or subgroups whose mediator values are more likely to change upon intervention). For example, Naimi, et al [218] defined A^* as women with less than high school education and C^* as women whose maternal age was <20.

In step three, redefining M only among those who were selected in step two. If M is a dichotomous variable, it can simply be set to the reference category to indicate effective

intervention on mediator. If it is a continuous variable with a normal distribution, redefinition can be realised with a random draw from a normal distribution [218]

$$M^* = f_N(M|A, C, L, R = 1;\ \xi)$$

where f_N represents normal distribution with a conditional mean $(M|A, C, L)$ and variance ξ.

In step four, outcome transformation for CDE_{RD} is achieved with

$$\tilde{Y} = Y - \beta_m(M - M^*) - \beta_{am}(A{\times}M - A{\times}M^*)$$

where β_m and β_{am} are parameters obtained in model 3 with identity link function. Outcome transformation for CDE_{RR} is achieved with

$$\tilde{Y} = Y\ \exp[-\beta_m(M - M^*) - \beta_{am}(A{\times}M - A{\times}M^*)]$$

where β_m and β_{am} are parameters obtained in model 3 with log link function [218].

In the last step, the transformed outcome model takes the form

$$E\left(\tilde{Y}\middle|A, C\right) = \gamma_0 + \gamma_a A + \gamma_c C \qquad (Model\ 4)$$

and CDE_{RD} can be obtained with identity link assuming normal distribution and CDE_{RR} with log link assuming log normal distribution.

Although the stochastic mediation contrast method reflects the real-world intervention scenario, there are certain situations where this method may have limitations. In a repeatedly measured cohort study, it is possible to have time-varying mediator-outcome confounders affected by prior mediator. For example, when studying the association between physical activity level (A in Figure 6.2.1) and blood pressure (Y in Figure 6.2.1), blood cholesterol level

may mediate part of the effect (M in Figure 6.2.1). Dietary habit (e.g. high fat and high salt intake) may confound the mediator outcome relationship (L in figure 6.2.1). Blood cholesterol level at prior visit may affect one's dietary habit (assuming that people may change their dietary habit when they are informed of their blood cholesterol level). Therefore, dietary habit can be a time-varying mediator-outcome confounder affected by prior mediator. In the presence of time-varying mediator-outcome confounder, direct conditioning on L, as shown in model 3, may bias the parameter β_m.

Here we explored an alternative approach which is built upon marginal structural model [176] and stochastic mediation contrast [218]. The implementation steps include,

1. $IPTW$ and $IPMW$ will be calculated based on methods illustrated previously [103,124,176].

2.Marginal structural model 2 will be fitted after each observation is weighted with their corresponding $SW = IPTW * IPMW$.

3. Fraction of observations will be selected using the method illustrated in the stochastic mediation contrast [218] with selection probability based on plausible assumptions regarding effectiveness of intervention on mediator.

4. Binomial outcome will be redistributed with random draw from a binomial distribution

$$\begin{cases} \Pr[Y_{am^*} = 1 | A = a, M = m^*] = \{1 + \exp(-(\alpha_0 + \alpha_1 a + \alpha_2 m^* + \alpha_3 a m^*))\}^{-1} & if\ R = 1 \\ Y_{am^*} = Y & if\ R = 0 \end{cases}$$

and continuous outcome will be redistributed with random draw from a normal distribution

$$\begin{cases} f_N[Y_{am^*}|A=a, M=m^*] \sim f_N[(\alpha_0+\alpha_1 a+\alpha_2 m^*+\alpha_3 am^*), \delta] & if\ R=1 \\ Y_{am^*}=Y & if\ R=0 \end{cases}$$

where Y_{am^*} is counterfactual outcome and $Y_{am^*}=[Y_a|Set\ M=m^*]$, α_n are parameters estimated from the marginal structural model 2, δ is variance of continuous outcome variable and R is a dichotomous variable indicating subject selection at step 3 (will take 1 if selected and 0 otherwise).

When exchangeability and consistency assumption hold [124], the counterfactual outcome Y_{am^*} will be independent of M. In other words, M is no longer a mediator in the association between A and Y_{am^*}.

5. Fit the following marginal structural model where each observation is weighted with $IPTW$ only

$$E(Y_{am^*})=\alpha_0+\alpha_1 a \qquad (Model\ 5)$$

to reduce simulation error and obtain stable estimates, step 4 and 5 need to be repeated, for example, 100 times or more. Mean α_1 across repetitions can be used as parameter of interest and mean SE_{α_1} can be used to construct its 95% CI.

Appropriate policy target for intervention on mediator may be obtained by repeating step 3-step 5 for different levels of intervention effectiveness on mediator as was shown previously [218]. The effectiveness of intervention at which the 95% CI of CDE crosses the null can be regarded as minimal policy target to eliminate exposure effect.

When the intervention on the mediator is 100% effective, the exposure effect α_1 in model 5 corresponds to CDE (precisely speaking, CDE when $M = 0$) because this 100% effective intervention will remove the arrow from M to Y in Figure 6.2.1 and M does not act as a mediator; when it is 0% effective, it corresponds to the total exposure effect. If the intervention effectiveness is >0% and <100%, α_1 does not correspond to CDE because part of the mediating effect of M contributes to this quantity. However, this quantity is exactly what policy makers needed to understand the required degree of intervention effectiveness on mediator to block detrimental exposure effect and to allocate appropriate resources on mediator intervention. In this paper, with a slight abuse of terminology, we still call this quantity as CDE even though it does not correspond to "direct" effect under suboptimal intervention effectiveness on mediator.

Data Analysis

Missing values were imputed using multiple imputation by chained equations [134], and created 20 imputed datasets. The following steps were implemented in each data set separately and the final point estimate and variances were combined across the 20 imputed datasets using Rubin's rules [139,222].

Based on consultation with literature and subject matter experts, C in Figure 6.2.1 was defined to include sex, educational level, marital status, injection drug use (IDU) in the past 6 months, past depression status, past HIV viral load (detectable vs non-detectable using 50 copies/mL as threshold) and restricted cubic spline for natural log transformed CD4 count measured at prior visit. When the mediator is depressive symptoms, L included past HIV viral

load and restricted cubic spline for natural log transformed CD4 count measured at prior visit; when the mediator is cART adherence, L included depression and HIV clinical stage. Since FI has three levels, multinomial logistic regression was used to obtain $IPTW$.

As opposed to assessing the effect of specific exposure pattern (e.g. exposed at all visits vs not exposed at all visits), our interest with the current cohort data was to assess the effect of FI measured at visit k on HIV viral load and CD4 count measured at visit k while setting fraction of observations' depression (or adherence) to alternative values. Therefore, $IPTW$ and $IPMW$ were not cumulatively multiplied over visits. Instead, only visit specific $SW = IPTW * PMW$ was obtained. It was assumed that temporal order between exposure, mediator and outcome was assured through the time window in which they were measured. Specifically, the HFSSM measured participant's food experience over the past 6 months [219]; the CES-D-10 measured depressive symptoms over the past week [131]; the ACTG Adherence Questionnaire measured adherence over the past four days [132,203]; CD4 count and HIV viral load measures reflect the moment of sampling.

Marginal structural model 2 was fitted by weighting each observation with SW. A logit link with binomial distribution was used for detectable HIV viral load; an identity link with Gaussian distribution was used for natural log transformed CD4 count. Since inclusion of the exposure-mediator interaction term did not alter the result, all estimations were conducted without the interaction term.

Fraction of observations were selected using the same method proposed previously (step two in mediation contrast method mentioned above) [218]. However, we did not relate

selection probability to any specific variables. Instead, we assumed that people with depressive symptoms (or poor adherence) were equally likely to be targeted for intervention regardless of their age, race, educational level or other characteristics (or intervention on mediator is equally effective regardless of these characteristics). Selected observations' mediator variable (depressive symptoms or adherence separately) was set to reference level to represent effective intervention on mediator.

Detectable HIV viral load was redistributed with a random draw from binomial distribution and log CD4 count was redistributed with random draw from normal distribution shown in implementation step 4 in previous section. Model 5 was fitted using redistributed outcome variables. Poisson regression model with log link was specified for detectable HIV viral load and a linear regression model with identity link was specified for log CD4 count. Sandwich variance estimator was used to account for within subjects clustering induced by repeated measurement and weighting.

In the current analysis, we repeated step 4 and 5 for 100 times to reduce simulation error and then repeated step 3 to step 5 for each discrete level of effectiveness from 0% to 100% to demonstrate how CDE changes with different level of intervention effectiveness. Non-parametric Lowess smoothing technique [223] was used for the distribution of point estimates and corresponding 95% CI across the range of 0%-100% effectiveness. However, a band width of 0.2 was chosen to avoid over smoothing and to show wiggles potentially caused by simulation random error.

Results

A total of 725 patients co-infected with HIV-HCV were enrolled in the FS Sub-study from November 15, 2012 to October 15, 2015. The majority were male (73%) and the median age was 49 years with an inter quartile range (IQR) of 43 and 54 years. The median HIV infection duration was 14.1 years (IQR: 8.3-18.4). There were 1973 person-visits accrued over three years of follow up, of which 1050 person-visits were FI. The number of patients completing each study visits were: 725, 608, 420, 203, and 17, respectively. The numbers of patients experiencing FI at each visit were: 57%, 52%, 50%, 48%, and 71%, respectively. Important sociodemographic, socioeconomic and clinical characteristics of study participants at baseline are presented in Table 6.2.1.

When presence of depressive symptoms was used as a mediator, the association between severe FI and detectable HIV RNA was gradually attenuated with the increased effectiveness of an intervention on depressive symptoms. However, the association was still statistically significant even when the mediating role of depressive symptoms was completely blocked with a hypothetical 100% effective intervention: people experiencing severe FI had 1.33 (95% CI: 1.02-1.74) times the risk of having detectable HIV viral load compared with people not experiencing any FI when all participants were not reporting any depressive symptoms. No clear pattern of CDE was observed when outcome was CD4 count and the CDE remained significant regardless of the effectiveness of an intervention on depressive symptoms. The changes of CDE of severe FI on detectable HIV viral load and CD4 count with continuous increase of intervention effectiveness on depressive symptoms and its pointwise 95% CI are provided in Figure 6.2.2 and Figure 6.2.3. When cART adherence was used as a mediator, similar pattern was observed as with depressive symptoms, a decreasing CDE of severe FI on

detectable HIV viral load with increasing effectiveness of intervention on cART adherence. The CDE of severe FI on HIV viral load was reduced to a borderline non-significant association (CDE=1.35, 95% CI:0.97-1.87) with a 100% effective intervention on cART adherence. The association between severe FI and CD4 count, on the other hand, remained significant throughout (Table 6.2.2).

Figure 6.2.4 displays the discrete change of intervention effectiveness on cART adherence from 0% to 100% on the X axis and the corresponding change of CDE of severe FI on detectable HIV viral load and its pointwise 95% CI on the Y axis. There is a monotonic decrease of CDE with the increase of intervention effectiveness on cART adherence and the 95% CI crossed the null when the intervention effectiveness is 75% or above. Figure 6.2.5 shows the change of CDE when CD4 count was used as an outcome. The CDE remained significant regardless of intervention effectiveness although a small decreasing trend was observed.

Discussion

Although inverse probability weighted marginal structural method [176] can correctly estimate CDE, it implicitly makes an unrealistic assumption regarding intervention effectiveness on the mediator variable. Stochastic mediation contrast [218], on the other hand, defines intervention effectiveness on the mediator based on real-world scenario and creates transformed outcome by factoring out the mediator effect. In this method, correctly identifying mediator effect is essential in transforming outcome. In a cohort study involving repeated measures, it is possible to have time-varying mediator-outcome confounders affected by prior mediator and its presence will require inverse probability weighting approach to correctly

quantify the mediator effect. Therefore, the regular regression approach may not be able to correctly quantify the mediator effect and thus the average of transformed outcome cannot be "thought of as the average outcome with the effect of the mediator removed" [218].

In this manuscript, we used a stochastic mediation analysis with an outcome redistribution approach to estimate the mediating roles of depressive symptoms and cART adherence in a 'real-world' scenario. Our results indicated that effective intervention on depressive symptoms and cART adherence attenuated the original total effect of severe FI on HIV viral load and shifted it towards the null. However, the remaining effect was still strong: people experiencing severe FI had 30% higher risk to have detectable HIV viral load compared with people who were not experiencing FI when they all have perfect cART adherence or none of them had depressive symptoms. This result reinforces the hypothesis that pathways other than behavioral, such as nutritional compromise may also play important role in the association between severe FI and HIV viral load. The amount of attenuation by intervention on depressive symptoms and cART adherence was very similar (Table 6.2.2), possibly indicating that both mediators lie on the same behavioral pathway sequentially. In other words, people may develop depressive symptoms when there is severe food shortage and then these depressive symptoms may disrupt their cART adherence, where suboptimal cART adherence is a predictor of detectable HIV viral load [42,46,47,143,157,214].

Although the same mechanism is true for the association between severe FI and CD4 count, we were unable to observe the mediating role of either depressive symptoms or cART adherence. Kinetic studies showed that potent inhibitors of HIV-1 reverse transcriptase and protease produce a rapid exponential decrease in plasma virus levels with a very short half-live

of viral decay (mean $t_{1/2}$ is 2.1 days) [14]. In other words, it is possible to reduce plasma viral load by half in 2.1 days if patient has perfect adherence and cART regimen is effective. On the other hand, the average absolute increase in CD4 count per day was calculated as 8 cells/uL under effective treatment [224]. These differences in reaction time may explain why the mediating roles of cART adherence and depressive symptoms were observed in the association between severe FI and HIV viral load, but not in the association between severe FI and CD4 count. The exponential decay of free HIV virions may have enabled the rapid response of HIV viral load when the cART adherence was set to perfect or the presence of depressive symptoms was set to absence. Conversely, the one week mediator-outcome interval was probably too short to have significant CD4 increase given its relatively slow speed of increase in absolute numbers [224]. Lagging both exposure and mediator by one visit to allow for longer mediator-outcome interval was attempted. However, the overall null association between exposure and outcome in the lagged analysis did not allow us to assess mediating effect of both mediators.

Our study has a number of strengths. First, the mediation analysis implicitly assumes a temporal order for exposure, mediator and outcome. The prospective collection of data and measurement window of variables in this study enabled us to assure the temporal order between them. Second, we used an analytical approach offering the benefits of both the marginal structural method [176] and the stochastic mediation contrast [218]. Application of the marginal structural model allowed it to be used in the presence of time-varying mediator-outcome confounders and the stochastic approach allowed it to mimic realistic intervention settings. This study has also several limitations. First, we cannot interpret our estimated association as causal. To claim causality, some untestable assumptions such as exchangeability,

consistency and positivity have to be met. In the causal mediation analysis, unlike other causal methods, it is required to have exchangeability for exposure and mediator simultaneously. In other words, it requires the potential outcome to be independent of both exposure and mediator given exposure-outcome and mediator-outcome confounders [176], and therefore, is equivalent to 'double randomization' of both exposure and mediator variable. Unfortunately, it is impossible to verify this assumption based on observed data. Second, reduction of random error and obtaining stable estimates require considerable amount of repetition of the outcome redistribution steps. This procedure has considerably increased computational burden when we searched for policy relevant cut-offs for intervention effectiveness on mediator. However, it should be noted that we examined every discrete level of effectiveness from 0% to 100% to demonstrate the change of CDE with increasing intervention effectiveness on mediators. Future study could conduct bisectional searching approach for the policy relevant cut-offs to reduce computational burden. For example, the CDE can be assessed when intervention effectiveness is 50%. If the CDE is significant, it can be recalculated by specifying intervention effectiveness as 75%, and so on. If, however, the CDE is not significant at 50%, then it can be recalculated by setting intervention effectiveness as 25%, and so on. This approach enables investigators to quickly find a point where the 95% CI of CDE crosses the null and this intervention effectiveness on mediator can be set as a target for eliminating harmful exposure effects on chosen outcome.

In conclusion, intervention on cART adherence and/or depressive symptoms was not sufficient to block the detrimental effect of severe FI on HIV viral load and CD4 count among people co-infected with HIV-HCV. Given the significant amount of direct effect, intervention on

FI must be accompanied to improve HIV related treatment outcomes among this population subgroup.

Table 6.2.1 Baseline characteristics by exposure status of participants in the Food Security & HIV-HCV Sub-study, Canada, 2012-2015

Characteristics	Total (N=725)	Food secure (N=309)	Food Insecure (N=416)		No. of missing values
			Moderate (N=170)	Severe (N=246)	
Age (years) [a]	49 (43, 54)	50 (44, 55)	48 (40, 53)	48 (43, 53)	2
Male (%) [b]	528 (73)	240 (78)	114 (67)	174 (71)	4
Caucasian (%)	542 (75)	237 (77)	119 (70)	186 (76)	10
Married or common law (%)	133 (18)	67 (22)	28 (16)	38 (15)	12
College or more education (%)	190 (26)	106 (34)	27 (16)	57 (23)	9
Gross monthly income below 1000 CAD (%)	308 (42)	106 (34)	72 (42)	130 (53)	6
IDU in the past 6 months (%)	238 (33)	60 (19)	62 (36)	116 (47)	21
Depressive symptoms (%)	338 (47)	89 (29)	81 (48)	168 (68)	8
Clinical AIDS (%)	43 (6)	20 (6)	6 (4)	17 (7)	2
On cART (%) [c]	662 (91)	289 (94)	160 (94)	213 (87)	0
Adherent (%) [d]	171 (26)	101 (33)	42 (25)	31 (13)	70
HIV infection duration (years) [a]	14.1 (8.3, 18.4)	15.3 (8.3, 20.0)	13.1 (8.5, 17.1)	13.5 (8.2, 17.4)	24
CD4 cell count (cell/uL) [a]	460 (300, 665)	491 (320, 692)	457 (290, 645)	440 (288, 630)	31
HIV RNA below 50 copies/mL (%)	547 (75)	248 (80)	127 (75)	172 (70)	44

a, median (inter quartile range); b, number (proportion); c, cART, combined antiretroviral treatment; d, sample was restricted to those who were taking cART.

Table 6.2.2 Controlled direct effect of severe food insecurity on detectable HIV viral load and CD4 count at different intervention effectiveness on depression and cART adherence among participants in the Food Security & HIV-HCV Sub-study, Canada, 2012-2015

Mediator	Intervention effectiveness on mediator	CDE [a] of Severe FI on Detectable HIV RNA	CDE of Severe FI on CD4 Count
Depressive symptoms	0%	1.47 (1.14, 1.88)	0.91 (0.84, 0.98)
	50%	1.39 (1.05, 1.83)	0.90 (0.83, 0.98)
	100%	1.33 (1.02, 1.74)	0.90 (0.83, 0.96)
cART [b] adherence	0%	1.48 (1.11, 1.97)	0.91 (0.84, 0.98)
	50%	1.43 (1.04, 1.96)	0.92 (0.84, 0.99)
	100%	1.35 (0.97, 1.87)	0.92 (0.85, 0.99)

a, CDE, Controlled Direct effect; b, cART, combined antiretroviral treatment.

Figure 6.2.1 Mediation with exposure A, mediator M, outcome Y, exposure-outcome, exposure-mediator and mediator-outcome confounders C and mediator-outcome confounders L.

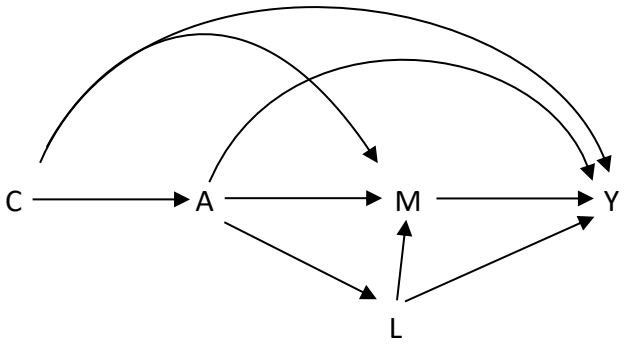


Figure 6.2.2 Changes of the controlled direct effect of severe food insecurity on detectable HIV viral load with different effectiveness of intervention on depressive symptoms among participants in the Food Security & HIV-HCV Sub-Study, Canada, 2012-2015

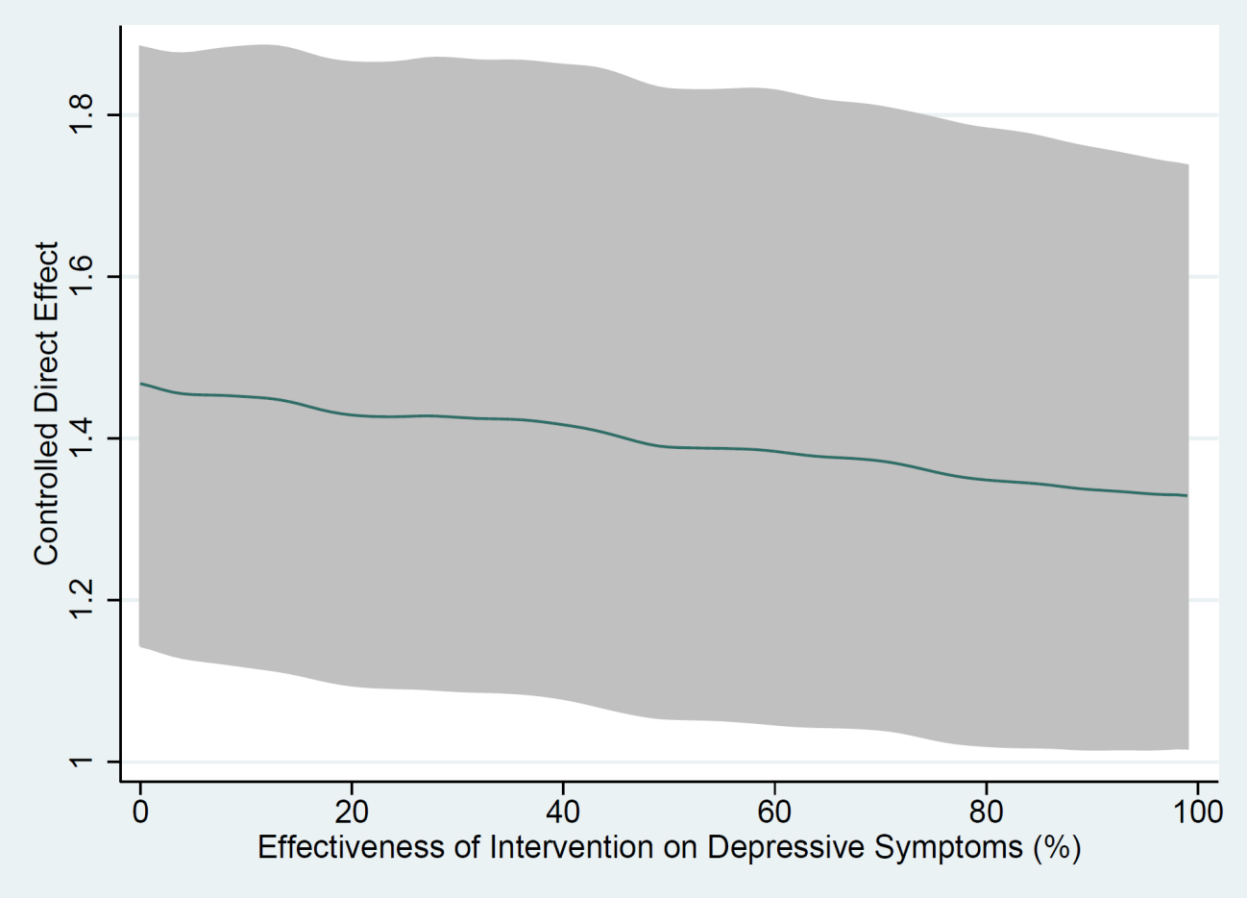


Figure 6.2.3 Changes of the controlled direct effect of severe food insecurity on CD4 count with different effectiveness of intervention on depressive symptoms among participants in the Food Security & HIV-HCV Sub-study, Canada, 2012-2015

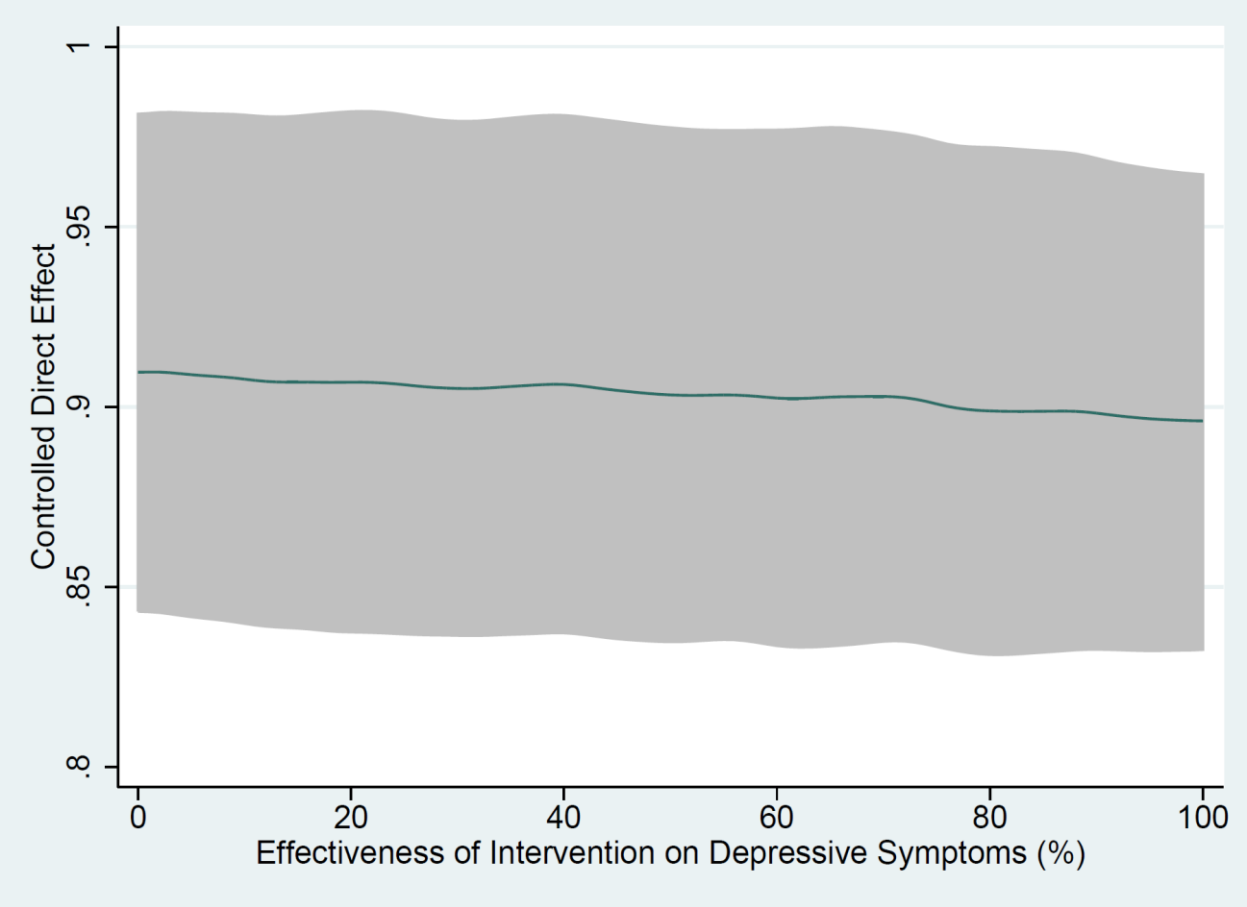
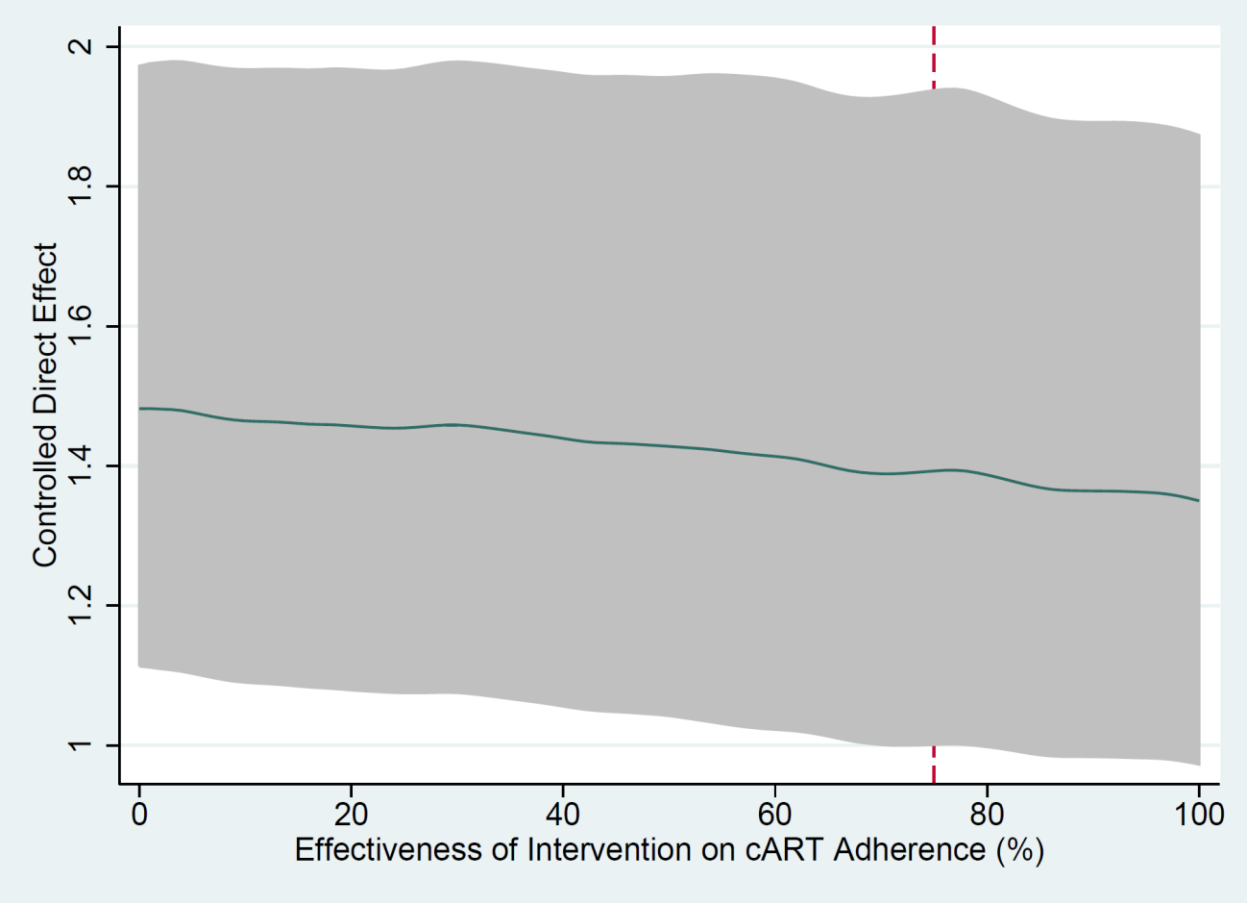
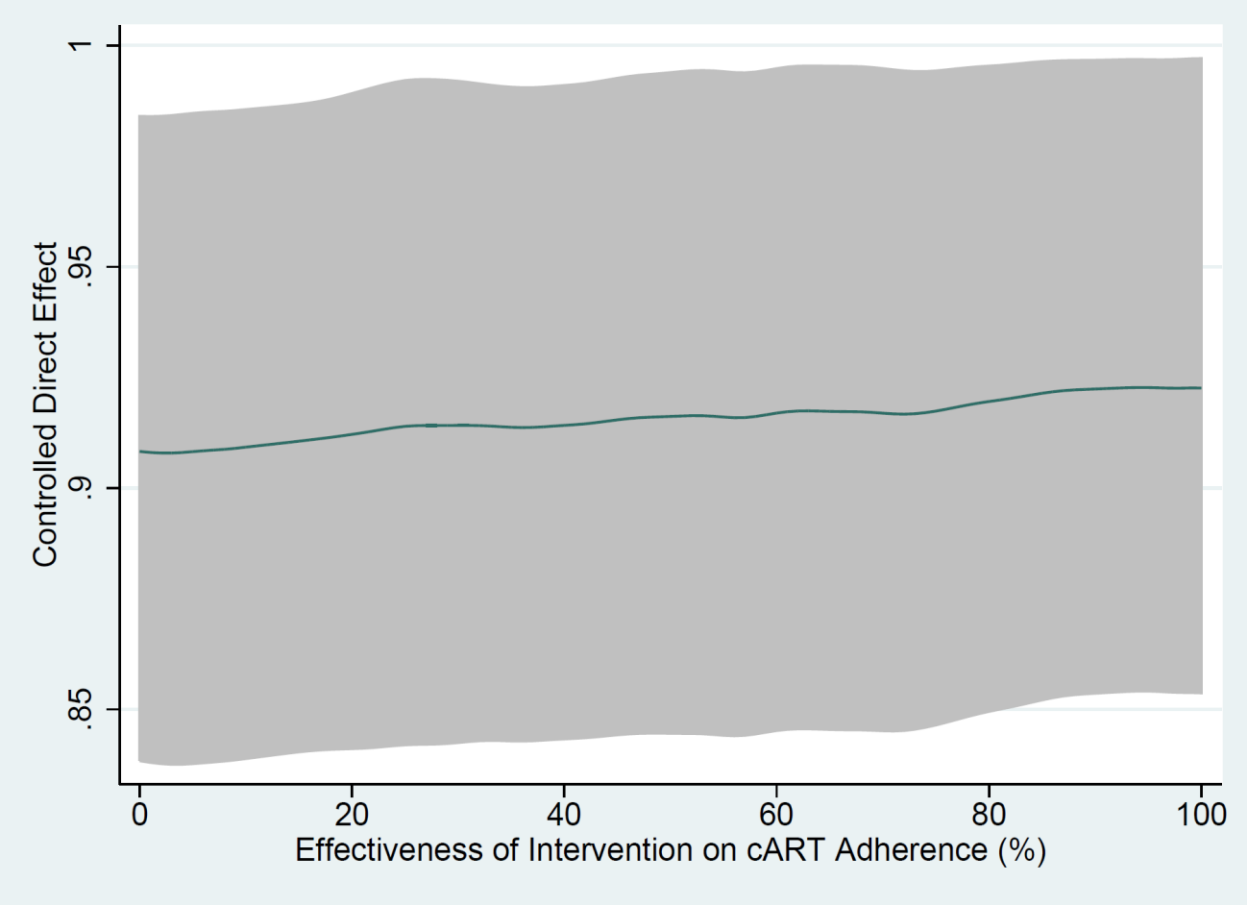


Figure 6.2.4 Changes of the controlled direct effect of severe food insecurity on detectable HIV viral load with different effectiveness of intervention on cART adherence among participants in the Food Security & HIV-HCV Sub-study, Canada, 2012-2015



Both controlled direct effect and 95% CI were smoothed with non-parametric Lowess smoothing technique with band width of 0.2.

Figure 6.2.5 Changes of the controlled direct effect of severe food insecurity on CD4 count with different effectiveness of intervention on cART adherence among participants in the Food Security & HIV-HCV Sub-study, Canada, 2012-2015



Both controlled direct effect and 95% CI were smoothed with non-parametric Lowess smoothing technique with band width of 0.2.

Chapter 7: Summary and Conclusion

7.1 Summary of Findings

The overall goal of this PhD thesis was to assess the association between food insecurity (FI) and HIV related health outcomes among HIV-HCV co-infected people, and to assess the mediating role of depressive symptoms in that association.

In systematic reviews and meta analyses (manuscript 1 and 2), it was shown that people experiencing FI had 32% more odds to have lower CD4 count and 29% less odds to achieve HIV viral suppression among HIV infected people with unknown proportion of HCV co-infection. In manuscript 3, the associations between FI and both HIV viral load and CD4 count were assessed exclusively among HIV-HCV co-infected people. We documented that experiencing severe FI, not moderate FI, was associated with increased risk of having detectable HIV viral load and lower CD4 count among co-infected people in Canada.

To assess the mediating role of depressive symptoms in the associations between FI and both HIV viral load and CD4 count, the first step should be to determine if the presence of depressive symptoms lies on the pathway between FI and HIV related health outcomes. For this end, manuscript 4 assessed the association between FI and the presence of depressive symptoms and demonstrated that both moderate and severe FI were associated with the presence of depressive symptoms in a dose response pattern. Manuscript 5 assessed the association between the presence of depressive symptoms and HIV related health outcomes. The results of indicated that the presence of depressive symptoms was associated with higher

risk of having detectable HIV viral load. However, the same association did not hold for CD4 count and we provided potential reasons for these discrepant findings.

Built upon findings from manuscript 3, 4, 5, a stochastic mediation analysis was conducted in manuscript 6 to assess the mediating role of depressive symptoms in the association between severe FI and HIV related health outcomes. The mediating role of cART adherence was also assessed for a comparison purposes although it was not listed as an original objective in the thesis project. The results of this manuscript showed that the total effect of severe FI on HIV viral load was decreased with the increasing effectiveness of intervention on mediators. However, considerable amount of direct effect remained even with a hypothetical 100% effectiveness intervention, indicating the existence of pathways other than depressive symptoms and cART adherence.

7.2 Strength and Limitations

The major strength of this thesis is the data source. The Food Security & HIV-HCV Sub-Study (FS Sub-study) of the Canadian Co-Infection Cohort (CCC) [120,219] was a prospective cohort study that followed HIV-HCV co-infected people biannually. Its inherent nature enabled the temporal order between exposure, mediator and outcome variables. The participants were recruited from the CCC, which is an ongoing cohort study that covers 18 treatment sites across six provinces in Canada. Recruitment sites are located in both large urban centres and smaller cities [120]. Based on the broad recruitment strategy, the sample may be representative of HIV-HCV co-infected people receiving care in Canada. In addition, as a prospective cohort study,

measurement of covariates was well planned. As a result, it was possible to consider many important confounders and to derive well-adjusted associations.

While biannual updating of information allows for more precise measurement of variables and reflects their change over time, it also creates difficulties in terms of analysis. Particularly, time-varying confounders affected by prior exposure may arise in cohort studies involving repeated measurement. Adjusting for time-varying confounders through conventional regression adjustment may introduce collider stratification bias, while not adjusting for it will confound the subsequent exposure-outcome associations. Throughout this thesis, inverse probability weighted marginal structural model was used to account for potential time-varying confounders.

Although exposure and mediator measurements were collected using questionnaire, all measurement scales had been validated in various settings and were widely used in the literature. Specifically, food insecurity (FI) was measured using the adult scale of the Canadian Community Health Survey's (CCHS) Household Food Security Survey Module (HFSSM); Depressive symptomatology was measured using the short version of the Center for Epidemiologic Studies Depression Scale (CES-D); and cART adherence was measured using items from the AIDS Clinical Trials Group (ACTG) Adherence Questionnaire. Therefore, these tools likely reflect valid measures of the identified constructs.

It is important also to recognize several limitations related to this thesis work. First, not causal relationships can be claimed. To have causal relationship, several assumptions such as the counterfactual consistency assumption and no unmeasured confounding must be upheld.

Given the observational nature of this study and the complex psychosocial constructs of the exposure and mediator variables, it is difficult to meet these conditions. We also acknowledge the possibility of residual confounding because the exposure was not 'allocated randomly'. Second, the presence of depressive symptoms was measured using the short version of the Center for Epidemiologic Studies Depression Scale. Since it is designed to screen for depressive symptoms among the general population, it is known to have higher sensitivity and lower specificity. Therefore, it may have captured transient and reactive depressive symptoms rather than a chronic clinical depression state, even though screening positive suggests a higher probability of having clinical depression. Third, no data was available on whether participants who reported depressive symptoms and poor cART adherence received any kind of counseling or intervention that could mitigate effects on HIV load and CD4 count. Receiving such counseling may have improved depressive symptoms and cART adherence before the next visit and have shifted the association towards the null. Lastly, although a sample size of 725 is quite large given the specific characteristics of this population, it is still limited in terms of statistical power for assessing certain dose response relationships.

7.3 Implications of Findings

Two systematic reviews and meta-analyses conducted as part of this thesis work are the first ones that summarised associations between food insecurity (FI) and both HIV viral load and CD4 count among HIV infected people. Although the results indicated that FI was associated with HIV viral load and CD4 count, it remained unclear if these associations would hold among HIV-HCV co-infected people. Results from the third manuscript served to fill this gap and

showed that FI was also associated with both HIV viral load and CD4 count among people co-infected with HIV-HCV. Since this thesis work did not assess the association between an improvement in food security status and HIV related health outcomes, it is difficult know whether improving food security status leads to improvement in HIV viral control and CD4 count. However, the results of this analysis may encourage health professionals and program managers to more actively consider FI and take efforts to improve food security status.

Manuscripts 4 and 5 mainly served as an assessment of the mediating role of depressive symptoms in the associations between FI and both HIV viral load and CD4 count. The results indicated that the presence of depressive symptoms did indeed lie on the pathway between FI and HIV viral load. The strong association between FI and depressive symptoms suggests that health professionals should consider FI as a potential reason for patients presenting with depressive symptoms, whereas the association between depressive symptoms and detectable HIV viral load could encourage more systematic depression screening.

Manuscript 6 assessed the mediating role of depressive symptoms and cART adherence in the associations between FI and both HIV viral load and CD4 count. The results of this analysis indicated that an intervention on depressive symptoms and cART adherence were unable to block the detrimental effect of FI on HIV viral load and CD4 count among HIV-HCV co-infected people. Therefore, intervention on FI must be accompanied to improve HIV related treatment outcomes if people were experiencing FI. Lastly, the methods used in this manuscript were novel and could be applied in other mediation analyses where the exposure may not be amenable to intervention.

7.4 Conclusion

Based on the results of this thesis, we can conclude that FI is a risk factor for having detectable HIV viral load and lower CD4 count among both HIV mono-infected and HIV-HCV co-infected people. In addition to poor HIV related health outcomes, FI also increases the risk of experiencing depressive symptoms among HIV-HCV co-infected people. Although presence of depressive symptoms is associated with incomplete HIV viral suppression, it is not associated with lower CD4 count. Intervention on depressive symptoms and cART adherence was not capable to eliminate the harmful effects of FI on HIV related health outcomes.

APPENDIX A: Questionnaire for Food Insecurity Measurement Used in the Food Security & HIV-HCV Sub-Study*

1. **Which of the following statements best describes the food eaten in your household since your last visit? Check only one.**
   - o You/Your household always had enough of the kinds of foods you wanted to eat.
   - o You/Your household had enough to eat, but not always the kinds of food you wanted.
   - o Sometimes you/your household did not have enough to eat.
   - o Often you/your household did not have enough to eat.
2. **Since your last visit, you/your household worried that food would run out before you got money to buy more. Was this…**
   - o Often true
   - o Sometimes true
   - o Never true
3. **Since your last visit, the food that you/your household bought just didn't last and there wasn't any money to get more. Was this…**
   - o Often true
   - o Sometimes true
   - o Never true

A BALANCED MEAL is a meal that contains items from at least 3 food groups (vegetables and fruit, grain products, milk and alternatives, meat and alternatives).

4. **Since your last visit, you/your household couldn't afford to eat balanced meals. Was this…**
   - o Often true
   - o Sometimes true
   - o Never true
5. **Since your last visit, did you ever cut the size of your meals or skip meals because there wasn't enough money for food?**
   - o Yes
   - o No

If no, skip the following A and B, go to 6

5a: [If YES above] How often did this happen?

- o Every month
- o Some months but not every month
- o Only 1 or 2 months

5b: [If Yes above] How many days per month on average was this happening? ☐
Days per month

6. **<u>Since your last visit</u>, did you ever eat less than you felt you should because there wasn't enough money to buy food?**
    - Yes
    - No
7. **Since your last visit, were you ever hungry but didn't eat because you couldn't afford enough food?**
    - Yes
    - No
8. **Since your last visit, did you lose weight because you didn't have enough money for food?**
    - Yes
    - No
9. **Since your last visit, did you ever not eat for a whole day because there wasn't enough money for food?**
    - Yes
    - No

9a: [If YES above], how often did that happen?

- Every month
- Some months but not every month
- Only 1 or 2 months

***** The first question is used for screening, and will not be included in actual score calculation.

APPENDIX B: The Center For Epidemiologic Studies Depression Scale (CES-D) 10-Item Version†

Below is a list of ways you might have felt or behaved. Please tell us on how many days you have felt this way DURING THE PAST WEEK.

	Rarely (< 1 day)	Sometimes (1-2 days)	Occasionally (3-4 days)	Most of the time (5-7 days)
1. I was bothered by things that usually don't bother me.	0	1	2	3
2. I had trouble keeping my mind on what I was doing	0	1	2	3
3. I felt depressed.	0	1	2	3
4. I felt that everything I did was an effort.	0	1	2	3
5. I felt hopeful about the future.	3	2	1	0
6. I felt fearful.	0	1	2	3
7. My sleep was restless.	0	1	2	3
8. I was happy.	3	2	1	0
9. I felt lonely.	0	1	2	3
10. I could not get "going".	0	1	2	3

† The numbers in above table is the corresponding scores a person will get according to his/her answers to each questions. E.g. if a person answers “Most of the time” to first question, he/she will be assigned 3, if answers “Rarely”, instead, he/she will be assigned 0 for that question, and move to ask second question. If a person answers “Most of the time” to fifth question, he/she will be assigned 0. If he/she answers “Rarely”, instead, he/she will be assigned 3 for that question, and move to ask sixth question. A person can only be on one of the four columns for each questions.

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