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Examining the Association Between Iron Deficiency and Hemoglobin A1c Among Females in The National Health And Nutrition Examination Survey, 2003–2008 Using Propensity Score Analysis

Helen Bisrat

ABSTRACT

Examining the Association Between Iron Deficiency and Hemoglobin A1c Among Females in The National Health And Nutrition Examination Survey, 2003–2008 Using Propensity Score Analysis

By

Helen Habte Bisrat

1/3/2018

INTRODUCTION: Hemoglobin A1C (A1C) is a common test used in the diagnosis of diabetes mellitus and for long-term glucose management. Within the literature, it has been shown that there may be association between iron deficiency (ID) and A1C. In this study, we analyzed observational data using propensity score analysis to further explore this association.

AIM: The aim of this study is to compare the association of A1C and ID among non-pregnant, non-diabetic women aged 12-49 without a history of chronic renal disease using various statistical methods to control for covariate imbalance.

METHODS: Data on 4,656 women obtained from the National health and Nutrition Examination Survey (NHANES) during the period 2003-2008 was used to compare four different statistical methods to examine the association between A1C and ID: unadjusted and adjusted multivariable logistic regression and two different propensity score analyses to impose covariate balance between those who were ID and not-ID.

RESULTS: The unadjusted crude odds ratio between ID and elevated A1C was 2.32 (95% CI: 1.75, 3.07); while the adjusted odds ratio was slightly attenuated [OR = 2.1 (95% CI: 1.55, 2.85)] after controlling for age, income, education level, race, BMI, smoking status, and 24-hour dietary recall of iron intake. Further adjustment for the propensity score in the logistic model yielded an odds ratio of 1.88 (95% CI: 1.41, 2.49) if the propensity score was treated as a linear variable and 1.92 (95% CI: 1.45, 2.55) if treated as a categorical variable using the quintiles propensity score.

DISCUSSION: This study confirms the presence of a statistically significant association between A1C and ID, and further suggests this may be a causal association. These findings may have implications for diabetes screening if ID causes shifts in A1C.

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by

Helen H. Bisrat

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by

Helen H. Bisrat

Approved:

Matt Hayat, PhD
Committee Chair

Maya Sternberg, PhD
Committee Member

_____
Date

Author's Statement Page

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___Helen Bisrat_____
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1. Introduction

The effects of chronic health conditions are of increasing concern in the United States (U.S). As of 2012 more than half of adults in the U.S are managing at least one chronic illness (Ward, Schiller, & Goodman, 2014). One of the most common and well-known chronic illness is diabetes mellitus (DM). DM is one of the leading causes of morbidity and mortality in the U.S with the prevalence of DM steadily increasing (Diagnosis and Classification of Diabetes Mellitus, 2010). In 2015 the National Diabetes Statistics Report from the Centers for Disease Control and Prevention (CDC) reported that 30.3 million people have diabetes in the U.S, which is approximately 9.4% of the U.S population (National Diabetes Statistics Report, 2017). Diagnostic tools for determining diabetes include a fasting plasma glucose (FPG), a 2-h plasma glucose level after a 75-g oral glucose tolerance test (OGTT), or hemoglobin A1C (A1C) (Diagnosis and Classification of Diabetes Mellitus, 2010). A1C has quickly become the gold standard for measuring long-term blood glucose concentrations and is the preferred method for both clinicians and patients in diagnosing and managing diabetes. DM is characterized by hyperglycemia resulting from defects in insulin function. This hyperglycemia is associated with increased risk of organ damage and dysfunction in the eyes, heart, kidneys, and blood vessels (Diagnosis and Classification of Diabetes Mellitus, 2010). Diabetes is the leading cause of kidney failure, lower-limb amputations other than those caused by injury, and chronic kidney disease (CKD) (CDC, 2011). Along with these debilitating conditions, diabetes has also been linked to other aliments like anemia.

Anemia is a common condition associated with diabetes, and it is estimated about 10 to 30% of diabetic individuals are anemic (Hosseini, Rostami, Saadat, Saadatmand, & Naeimi, 2014). Individuals with CKD are also known to be anemic, and this knowledge is often correlated as

one of the reasons why diabetics tend to be anemic. However, studies have shown diabetes is associated with anemia outside of the development of CKD. In 2002, the Third National Health and Nutrition Examination Survey (NHANES-III) reported individuals with diabetes were nearly twice as likely to have anemia compared to individuals without diabetes who have a similar degree of renal impairment (Astor, Muntner, Levin, Eustace, Coresh; 2002). The association of DM and anemia is the product of multiple factors including nutritional deficiencies, diabetic medication, and impaired hormone production. Individuals with certain types of DM are at a higher risk of developing tissue-specific autoimmune diseases (McGill & Bell, 2006). Such autoimmune diseases can lead to malabsorption of iron into the body and cause nutritional deficiencies such as anemia (McGill & Bell, 2006). Additionally, certain medications used to treat diabetes can decrease hemoglobin concentrations; these drugs include angiotensin-converting enzyme inhibitors, and angiotensin receptor blockers (Ajmal, Gessert, Johnson, Renier & Palcher, & 2013). Lastly, erythropoietin (EPO) is a hormone produced and regulated by the kidneys that stimulates red blood cell production in the body. Individuals with DM commonly suffer from renal function impairment, and this affects the regulation and production of EPO leading to anemia (McGill &Bell, 2006).

The clinical relevance of the association between DM and anemia still needs to be studied and understood. Studies have shown that multiple forms of anemia are associated with lowering A1C concentrations, however, anemia caused by iron deficiency (ID) may have falsely elevated A1C concentrations, independent of chronic glycemia (Ahmad & Rafat, 2013). ID is the most common nutritional deficiency worldwide, and is the result of a long-term decline of iron level in the body (WHO, 2012). Its prevalence is highest among women of childbearing age, making this cohort disproportionately affected by potential misleading A1C results. One study by Kim,

Bullard, Herman, & Beckles (2010) found that the presence of ID is associated with shifts in A1C distribution to higher concentrations. Hong et al. (2015) also showed ID shifting levels of A1C upwards compared to non-iron deficient (NID) individuals. These studies provide practical evidence that an association exists between A1C and ID. Because A1C is a commonly used marker for glycemic control and diagnosis of diabetes, it is important to determine the degree of the causal effect ID on A1C to ensure proper diagnoses and treatments to patients. Previous studies that examined the association between A1C and ID are primarily observational and have not used statistical methods to assess an unbiased estimation of a causal effect between ID and elevated A1C. While randomized control trial (RCT) are gold standard to assess causal effects there are no such studies examining the relationship between ID and A1C due most likely to ethical and logistical considerations However, there have been advances in statistical methods over the last 30 years to examine causal relationships in observational studies. The most common of these methods is propensity score analysis.

Propensity score analyses have gained wide popularity as a statistical approach to assess a causal relationship between an exposure and outcome in an observational study. For the purpose of this study, the propensity score is the probability that a subject is ID based on that subject's characteristics (socio-demographic, behavioral etc). The idea is to use the estimated propensity score as an adjustment to ensure that the conditional distribution of characteristics is the same for ID and NID groups. There are several different ways propensity scores can be used: creating a matched sample of ID and NID who have similar propensity scores, stratify subjects on their propensity score and estimate the effect of elevated A1C within each strata, or including propensity score categorically or continuously as a predictor along with exposure in a the model

for the outcome of interest. This thesis will use a propensity score model treating the propensity score as a linear covariate and as a qualitative variable categorized along the quintiles.

The aim of this study is to examine the distribution of A1C by ID status among non-pregnant, non-diabetic women aged 12-49 without a history of chronic renal disease using a recent population-based probability sample of the United States and compare the estimated odds ratio using different statistical methods to control for covariate imbalance.

2. Literature review

The literature was searched within the year range of 2002 to 2017 to review the current state of the literature on the association of A1C and iron deficiency. Using the following search terms: (1)'HbA1c', 'Hemoglobin a1c', 'A1C', 'Glycohemoglobin' and (2) 'Iron deficiency,' 'anemia,' 17 articles were found that look at the association between A1C and ID using the criteria described above.

There are many observational studies exploring the association of ID on a patient's A1C level, none of which have previously used propensity score analysis. The preponderance of the literature supports the notion of a statistically significant association between ID and A1C, though there are some studies that have failed to find the association.

Among most of the smaller cross-sectional studies, individuals with iron deficiency are more likely to have higher A1C levels than those without iron deficiency. These studies had many aspects in common, including exclusion criteria used and patient population. Exclusion criteria in the majority of the cross-sectional studies more or less included patients without a history of chronic or acute blood loss, hemolytic anemia or haemoglobinopathies, kidney disease, chronic alcoholism, or impaired glucose tolerance. Diabetics and pregnant women were also excluded from a majority of the studies unless the group was of particular interest in the study. Rajagopal, Ganapathy, Arunachalam, Raja, & Ramraj (2017) and Shanthi, Revathy, Devi, & Subhashree (2013) both examined a non-diabetic population that included males and females. In both of the studies, their iron deficient group had higher levels of A1C than the non-iron deficient group. Hashimoto et al. (2008) and Koga, Saito, Mukai, Matsumoto, & Kasayama (2010) looked at pre-menopausal women and pregnant women, and both saw an increase in A1C levels within people that were ID. Silva, Pimentel, & Camargo (2015) and Rajagopal et al.

(2017) measured the association of iron deficiency and A1C on varying levels of anemia. These two studies both observed that varying degrees of iron deficiency is associated with A1C and that as the severity of anemia increased, so did A1C levels. However, Silva et al. (2015) did have varying results with patients with mild anemia, defined in this study as females with hemoglobin levels ≥ 11 g/dl and <12 g/dl and males with hemoglobin levels ≥11 g/dl and <13 g/dl. These researchers observed no significant difference between A1C values in participants without anemia and within the mild anemia group. Silva et al. (2015) conducted their study using two different methodologies for measuring A1C. Both ion exchange high-performance liquid chromatography (HPLC) and immunoturbidimetry revealed a statistically significant association with elevated of A1C values in the ID group compared to the non-ID group. However, while the majority of research supports the notion that ID is associated with elevated A1C measurements, there was a study by Kalasker, Sudhamadhuri, Kodliwadmath, & Bhat, (2014) that observed an association in the opposite direction. A possible explanation for this discrepancy compared to the other studies may be the methodology for measuring A1C, which was not well described in the paper.

Larger nationally representative cross-sectional studies have also been used to investigate the association of iron deficiency and A1C. Hong et al. (2015) used data from the Korea National Health and Nutrition Examination Survey (KNHANES) and Ford, Cowie, Li, Handelsman, & Bloomgarden (2010), Cheung C., Cheung T., Lam, & Cheung B. (2012) and Kim et al. (2010) used data from the U.S National Health and Nutrition Examination Survey (NHANES). Hong et al. (2015), Cheung et al. (2012), and Kim et al. (2010), all found the presence of iron deficiency was associated with shifts in A1C distribution to higher levels. Hong et al. (2015) and Kim et al. (2010) express that these findings were specific to certain A1C cutoffs. Hong et al. (2015) found

statistically significant differences in A1C levels of >5.7% and ≥ 6.1% but not ≥6.5%. Kim et al. (2010) observed similar results with the association in shifts in A1C distribution to higher levels occurring primarily between <5.5% and 5.5– 6.0%. Ford et al. (2010) also observed this trend; however, they saw participants with iron deficiency anemia (IDA), the most common form of iron deficiency, had similar A1C concentrations as participants with normal hemoglobin levels and normal iron status. Thus, Ford et al. (2010) concluded that iron deficiency anemia has little population effect on concentrations of A1C or diabetes prevalence. The Cheung et al. (2012) study focused more on the association of different body iron stores and pre-diabetes (preDM). Within their study they found that high serum ferritin level and low transferrin saturation were associated with pre-diabetes, contradicting the Ford et al. (2010) study results.

In addition to the cross-sectional designs there have been a few experimental longitudinal study design exploring the relationship between ID and A1C. These studies compared A1C levels before and after receiving iron supplements, either orally or intravenously. One of the very first studies to examine the association between A1C and ID was by Brooks, Metcalfe, Day, & Edwards (1980). Brooks et al. (1980) conducted an experimental longitudinal study comparing the change in A1C after receiving iron supplementation. Within this study Brook et al. (1980) found the mean A1C level was higher among ID participants. After receiving treatment, people who were iron deficient saw a significant decrease in their A1C concentrations. The majority of studies conducted recently (Coban, Ozdogan, & Timuragaoglu, 2004; El-Agouza, Shahla, & Sirdah, 2002; Hashimoto et al., 2008; Madhu, Raj, Gupta, Giri, & Rusia, 2017; Ng, Cooke, Bhandari, Atkin, & Kilpatrick, 2010; Rafat, Rabbani, Ahmad, & Ansari, 2012) found that A1C levels were also higher in participants with iron deficiency, and, once these participants were treated with iron supplements, their A1C levels decreased significantly,

similar to Brook et al. (1980). However, a study by Sinha, Mishra, Singh, & Gupta (2011) found conflicting results; this study saw that their baseline A1C levels in their iron deficient group were higher than their control group. After two months of iron supplementation, Sinha et al. (2011) saw A1C levels increased in the iron deficient group. This contrasting result could be attributed to a few factors in this study including study population and methodology. The patient population for Sinha et al. (2011) was all from a hospital from Delhi India, a potentially biased sample due to convenience sampling and the harsh socio-economic factors of that population. Instrumentation bias is a potential contributor to the observed results because the methodology for measuring A1C in the Sinha et al. (2011) study was by a glycohemoglobin reagent kit. All other longitudinal studies chose to use a standardized method of HPLC (Rafat et al., 2012; Madhu et al., 2017) or ion exchange chromatography (El-Agouza et al., 2002; Ng et al., 2010). Another limiting factor of the Sinha et al. (2011) study was the length of the study; because it was only carried out for approximately 60 days, whereas the other longitudinal studies had a longer follow-up duration between the baseline and final A1C measurement (Rafat et.al, 2012; Madhu et. al., 2017; Coban et. al., 2004). The shorter period may not have allowed sufficient time for body iron stores to be replenished.

As A1C continues to grow in popularity as a widely used marker of chronic glycaemia, additional research is needed to determine the effect of ID on A1C. Within the literature we see somewhat consistent results within the different study designs on the association of A1C and ID. However, the findings have not been universal and there seems to be additional caveats to this association depending on the severity of the anemia or the association being limited to a specific A1C cutoff. All the earlier studies have been observational and none of the statistical analyses used a propensity score approach. Some of the contradictory findings may be due selection bias

and/or covariate imbalance, which may be another reason for some of the conflicting findings. The purpose of this study is to examine the association between ID and A1C using a nationally representative probability sample using propensity score analysis in order to obtain covariate balance and mimic a randomized control trial.

3. Methods

3.1 Design and Procedure

In 1999, NHANES was redesigned to become a continuous survey without a break in between cycles. The NHANES are a series of cross-sectional national surveys of health and nutritional status conducted every two-years by the National Center for Health Statistics (NCHS) of the U.S. Centers for Disease Control and Prevention (CDC). These surveys obtain a nationally representative survey of non-institutionalized individuals based on a complex, multi-stage probability sampling design. Data collected from NHANES is extensive and includes personal interview questions, physical examinations, and laboratory studies. Protocols for conducting NHANES were approved by the NCHS review board and informed consent was obtained from all participants.

The general sampling design of NHANES complex sampling can be described through four stages of sampling. First, the primary sampling units (PSUs) are selected, which are usually counties or groups of counties with probability proportional to a measure of size (PPS). Second, the selected PSUs are divided into segments, equivalent to city blocks, and selected with PPS. In the third stage households within each segment are randomly selected with unequal probabilities of selection to over sample certain subgroups of people, such as adolescents, African-Americans, and Mexican Americans. In the fourth stage individuals are chosen to participate in NHANES from all individuals living in the selected households, in the selected segments, in the selected counties. Individuals are drawn at random within selected subdomains (age, sex, race) (NHANES - Continuous NHANES Web Tutorial - Survey Design Factors).

Two-year interview and medical examination survey weights are provided by NHANES to account for the unequal probabilities of selection and adjustment for non-response across all

the stages of sampling. Analysts are recommended to use both the sampling weights and survey design variables for analyses. Failure to follow these recommendations can lead to biased estimates and incorrect standard errors leading to statistical inference that fails to have the stated coverage (or error rate) (Lohr, 2010).

3.2 Propensity score

Propensity score analyses is used to assess the degree of a causal relationship between an exposure and outcome in an observational study. This is achieved by fitting two separate statistical models. The first statistical model is used to estimate the propensity scores. The propensity score is the probability of being exposed conditional on observed baseline characteristics (Austin, 2011). The propensity score, if properly constructed, can work as a balancing score, to create groups that have similar distribution as determined by the covariates included in the model (Austin, 2011). We define the propensity score in this study as the conditional probability that women aged 12-49 years old would have ID given a set of selected observed covariates; this can be expressed as:

$$e(x)=Pr[E=1|x]$$

where e(x) is the propensity score, E is iron deficiency (exposure), and x is the vector of covariates. The second statistical model is used estimate unbiased causal effects by utilizing the estimated propensity scores based on the first model.

An unbiased causal treatment effect using propensity scores methodology is possible if certain assumptions are satisfied. These assumptions include: positivity, consistency, stable unit-treatment-value assumption (SUTVA) and strong ignorability. If these assumptions are met, then conditioning on the propensity score allows for unbiased estimates of the average exposure effect (Rosenbaum and Rubin, 1983). Of course, the propensity score is highly reliant on all

confounders being accounted for in the model; the balancing score and any analyses using the propensity score will be biased if this is not held true (Rosenbaum and Rubin, 1983).

3.3 Participants

Starting in 2003, iron measurements in NHANES were limited to children (1-5 years) and women of childbearing age (12-49 years). For this reason, only non-pregnant, non-diabetic women aged 12-49 without a history of chronic renal disease examined in the mobile examination center (MEC) from NHANES 2003-2008 with no- missing A1C and iron measurements are included in the analyses.

Variables used for exclusion criteria.

Variables selected for exclusion criteria included diabetes status, pregnancy status, and CKD. Diabetes status was determined through the personal interview questionnaire or fasting plasma glucose level ≥ 126. If the participant stated they were told by a doctor they were diabetic or they were found to have a fasting plasma glucose level ≥ 126 they were excluded from the study. Pregnancy status was also determined through the personal interview questionnaire and by a separate urine pregnancy test. Women who responded yes to being pregnant or had a positive urine pregnancy test result were excluded from the study. Chronic kidney disease was determined using Glomerular filtration rate (GFR). GFR is method to determine an individual's level of kidney function. This rate was calculated using an individual's serum creatinine levels and varies based an individual's age, gender, and race. An individual was considered to have CKD in this study if an individual's GFR fell below 60 mL/min (Andrew et al., 2006).

3.4 Study Variables

Dependent variable

A1C measurement was conducted using HPLC, a certified method by the National Glycohemoglobin Standardization program. In NHANES 2003-2004 an automated glycohemoglobin analyzer by Primus was used to determine percent A1C concentrations. In NHANES 2005-2006, a Tosoh A1C 2.2 Plus Glycohemoglobin Analyzer was used to determine percent A1C concentrations. In NHANES 2007-2008, A1C measurements were performed on the A1C G7 HPLC Glycohemoglobin Analyzer also measured in percent A1C concentrations. Although different A1C laboratory instruments were used between 2003 and 2008, crossover studies were conducted to ensure standardization throughout the years (NHANES 2005-2006: Glycohemoglobin Data Documentation, Codebook, and Frequencies.). For the purposes of this study, A1C was categorized into <5.5% and ≥5.5% as done in a previous study of this kind (Kim et al., 2010). Additional analysis on A1C categorized into <6.5 and ≥6.5% could not be conducted due to small sample size in the ID group.

Primary Independent variable of interest

The CDC laboratory measured serum ferritin and serum soluble transferrin receptor during 2003-2008. These biomarkers can be used to estimate body iron stores using an equation developed by Cook, Flowers, & Skikne (2003) based on the ratio of serum transferrin receptor to serum ferritin. The body iron equation developed by Cook et al. (2003) is less affected by inflammation than the previous models used, making it a more reliable measure of body iron stores. The formula developed by Cook et al. (2003) allowed for a participant's body iron stores that is < 0 mg/kg to be considered iron deficient. In this study we applied this formula to calculate body iron stores and subsequently categorize women into either ID or NID.

Other Covariates

All variables previously considered in the literature, as long as the variable was readily available in NHANES were selected for these analyses. The following covariates were used: age, race (Mexican American, Other Hispanic, Non-Hispanic White (NH white), Non-Hispanic Black (NH Black), Other Race), education level (less than high school, high school, more than high school), class income (lower class $0-$24,999, middle class $25,000- $54,999, upper middle class $55,000-$74,000, upper class $75,000), ratio of family income to poverty (PIR) (above and below poverty line), Body Mass Index (BMI) (<18.5kg/m2 (Underweight), 18.5 – 24.9 kg/m2 (Normal), 25.0 – 29.9kg/m2 (Overweight), >29.9kg/m2 (Obese)), recommended dietary allowance (RDA) iron intake using iron supplements (≥18 mg/day), 24-hour dietary recall of iron intake (mg), smoking status (smoked at least 100 cigarettes in life). Data was checked within all three cycles to ensure variable names matched and the question asked within the survey was the same.

3.5 Statistical Analyses

Statistical analysis was performed using SAS 9.4 software (SAS Institute Inc., 2015), including merging, cleaning, and recoding data.

3.5.1 Descriptive Statistics

Bivariate descriptive statistics were used to compare the selected covariates as well as the primary outcome A1C between ID and NID. SAS SURVEY procedures were used to account for the weighting, stratification, and clustering used in the survey study design. Arithmetic and geometric means were calculated for symmetric and skewed right continuous variables, respectively. P-values comparing the means are based on a Wald F test from a simple linear regression model using SURVEY REG. P-values comparing the distribution across categorical

variables between ID and NID are based on the Rao-Scott Chi-Square, the default statistic in SURVEYFREQ.

3.5.2 Multivariable Logistic regression

All selected covariates, regardless of statistical significance were included in the multivariable model to provide a fair comparison to the propensity score models. PROC SURVEYLOGISTIC was used to measure the strength of association between predictor and response variables by producing unadjusted and adjusted odds ratios and confidence intervals. Because a complex sample design will affect standard errors of the logistic regression coefficients, weights and survey design variables were accounted for (Lohr, 2010).

3.5.3a Propensity score model

The propensity score was estimated using an unweighted logistic regression. The propensity score model included: age (categorized into age groups), class income, education level, race, smoking status, RDA iron intake using supplements, 24-hour dietary recall of iron intake, BMI and the MEC survey weights. Since goal of the propensity model is not to provide inference to the U.S non-institutionalized population, the propensity model was not weighted nor were the complex survey design variables used. The goal of the propensity model is to make the exposed (ID) and unexposed (NID) as similar as possible. Therefore, the MEC survey weight is included as a covariate to improve the assumption of unconfounded treatment assignment (DuGoff et al (2014). Once the propensity scores were estimated from the propensity model, the scores were used either as a continuous variable or a categorical variable in the final model to estimate the association between ID and A1C (stratified into quintiles). An advantage of including the propensity score as an independent variable in a multivariable logistic regression model allows

inclusion of both exposed and unexposed individuals that would have been lost using other methods such as matching (Okoli, Sanders, & Myles, 2014).

3.5.3b Treatment effect propensity models

To estimate the causal effect of ID on HA1C, a PROC SURVEYLOGISTIC was to adjust for the propensity score. Two separate weighted logistic models were fit, one which treated the propensity score as a continuous variable and the other as a categorical variable, stratified into quintiles. Both models are survey weighted and account for the design variables in the variance estimations in order to estimate the population-level effect between ID and A1C, and compare it to the earlier unadjusted and adjusted odds ratio.

4. Results

4.1 Descriptive statistics

A total of 7,156 women aged 12-49 years old participated in the NHANES between 2003 and 2008. After excluding participants who were pregnant, diabetic, had CKD, and did not have measurements for A1C or body iron, 4,656 participants remained. Of the 4,656 women, 4,122 (88.50%) were considered NID and 534 (11.00%) were ID based on their calculated body iron stores (body iron stores <0 considered ID). The weighted descriptive statistics of participants' characteristics are displayed in Table 1. Women with ID had significantly higher A1C levels compared to women who were NID (5.31 vs. 5.15, p= <0.01). When comparing ID and NID women across various socio-demographic and behavioral factors there were statistically significant differences in age, smoking status, race, pre-diabetes status, iron supplements use, meeting RDA for iron supplementation, 24-hour dietary recall for iron intake, and PIR (Table1). Iron deficient women were significantly older than NID women (31.3 vs. 32.86, p = <0.01). The ID women were also less likely to be smokers compare with NID women (18.6% vs. 26.1%, p=0.01). The NID group breakdown of race included mostly NH white (65.20%) and NH black (12.26%), followed by Mexican American (9.26%). Within the ID group, the majority of participants were NH white (52.80%), NH Black (20.07%), and Mexican American (13.51%) (p=<0.01). Women in the ID group were also more likely to be pre-diabetic (12.63% vs. 5.29% p=<0.001). The percent of women in the ID group who took daily iron supplements was less than the percent of women in the NID group (20.63% vs. 31.28%, p= <0.01). Of the women who took iron supplements women in the ID group were also less likely to meet the RDA of iron supplementation compared to women in the NID (12.09% vs. 21.09%, p= <0.01). The mean 24-hour dietary recall of iron intake for the ID group was less than the mean 24-hour dietary recall

of iron intake for women in the NID (11.20 vs. 12.08, p=<0.01). Women in the ID group had a higher PIR rate than women in the NID group (2.47 vs. 2.09 p=<0.001). There was no statistical significance comparing ID and NID women across education levels, income classes, and BMI.

4.2 Association of ID and A2C using logistic regression analysis

Both the crude and adjusted weighted odds ratios suggest the odds of having A1C ≥ 5.5% is approximately twice the odds among NID women. The estimated crude weighted odds ratio between A1C (≥5.5%) and ID was 2.32 (95% CI: 1.75, 3.07). After controlling for age, income, education level, race, BMI, smoking status, 24-hour dietary iron intake, RDA of iron using iron supplementation the adjusted weighted odds ratio was 2.1 (95% CI: 1.55, 2.85)(Table 2). Similar to the descriptive analysis, the multivariable analysis showed younger women are significantly less likely to have an A1C level ≥5.5% than older women (p-value= <0.001). Specifically, women aged 12-19 had an odds ratio of 0.244 (95% CI: 0.156, 0.382) compared to women aged 40-49 at having an A1C level ≥5.5%. Women aged 20-39 had an odds ratio of 0.392 (95% CI: 0.311, 0.494) compared to women aged 40-49 at having an A1C level ≥5.5% (Table 2). Both Mexican-Americans and Non-Hispanic blacks had higher odds of being at an A1C level ≥5.5% compared to NH white women and found to be statistically significant. Mexican American women had an odd ratio of 1.735 (95CI%: 1.173, 2.567 and NH black women had an odds ratio of 2.487 (95CI%: 1.649, 3.752). One unit increase in BMI was found to increase the odds of having an A1C level ≥5.5% by 1.098 (95% CI: 1.077, 1.120). Within the multivariable logistic regression model smoking status, RDA of iron using iron supplementation, and 24-hour dietary recall of iron intake were no longer found to be statistical significant.

4.3 Propensity score

A logistic regression was used to estimate the propensity score for ID with the following covariates in the model: age, income, education, smoking status, RDA iron using supplementation, BMI, ethnicity, 24-hour dietary recall of iron intake and MEC survey weight. Based on this logistic model the mean of the estimated propensity scores among ID was 0.13 (standard deviation 0.47) and 0.11 (standard deviation 0.42) for NID. Table 3 summarizes the proportion of women who had an elevated A1C ($\geq$ 5.5%), as well as the mean propensity score among the ID and NID groups according to quintiles of the propensity score. There were 133 out of 443 women with ID who had A1C levels $\geq$5.5% and 588 out of 3,543 women with NID who had A1C levels $\geq$5.5%. Additionally, those with ID tended to have increased proportions of individuals with a higher propensity score compared to NID individuals. The ID group had approximately 65% of its individuals in the top 2 quintiles of the mean propensity score, while the NID had approximately 49% of its individuals in the top 2 quintiles of the mean propensity score.

The histograms and estimated probability density functions of the propensity scores for ID and NID are shown in Figure 1. The distribution of the propensity score controlling for the selected covariates, illustrates approximately equal overlap in probability estimates for both groups (Figure 1), ensuring the assumption of positivity is reasonable. There are only slight shifts seen in the tails of ID and NID, where the probability estimates in ID group shifted more towards 1 and the probability estimate in NID group shifted more towards 0.

4.4 Comparison of different methods

The odds ratios resulting from the different propensity score strategies are summarized in Table 4. Both approaches found a statistically significant association between A1C levels and ID. When using the propensity score as a continuous variable or as a categorical (quintiles)

variable the estimated adjusted odds ratios were comparable 1.88 (95% CI: 1.41, 2.49) and 1.92 (95% CI: 1.45, 2.55), respectively. The propensity score used as a continuous variable yielded the smallest estimated odds ratio, followed by the propensity score used as a categorical variable. The crude odds ratio displayed the largest estimated odds ratio.

5. Discussion/Conclusion

5.1 Discussion of Research Question

The aim of this study was to investigate the effect between ID and A1C among non-pregnant, non-diabetic women aged 12-49 without CKD, using propensity score analysis and compare these results to traditional logistic regression methods. Using NHANES 2003-2008 data, this study further confirms the association that ID is associated with increased shifts of A1C concentration utilizing both multivariable logistic models and propensity score models. The magnitude of the estimated association between ID and A1C across the various models is roughly similar. Each model shows that the odds among ID women of having an A1C $\geq$ 5.5% is approximately two times the odds of NID women. The possible improvement of the propensity model, if correctly specified, is this association can be interpreted as causal. In other words, if a NID women became ID we could expect that this change in her ID status would cause an increase in AIC. This type of interpretation is only possible with a statistical method that attempts to assign ID status such that those who are ID verses NID are as similar as possible, such that we can we can view ID status as being randomly assigned. It is not unreasonable to expect this association to be causal. Red blood cells must interact with glucose to form HbA1C, so underlying conditions that impact red blood cell turnover, such as ID, will affect HbA1C. While, the estimated odds ratio using propensity score analysis is slightly attenuated compared to multivariable logistic regression, the confidence intervals from the propensity models contain the

estimated odds ratios from the traditional logistic models. Therefore, the propensity model does not lead to a different inference than the traditional logistic models. However, this analysis adds to the literature by analyzing the effect between A1C and ID using methods that allows for unbiased estimates of causal exposure effect. The direction of the associations found in this study coincide with results from Hong et al. (2015) and Kim et al. (2010), who also found statistically significant differences in A1C levels in iron deficient women. In this study, we found a stronger association between ID and A1C among adult women than Kim et al. (2010) (OR: 1.33, 95% CI: 1.05–1.67) who also used NHANES data from 1999-2006 but only adjusted for age, race, waist circumference, parity and hysterectomy. This study contradicts the results of Ford et al. (2010) who concluded that adults with iron deficiency anemia had similar A1C concentrations as adults with normal iron status and normal hemoglobin using data from NHANES 1999-2002 after controlling for age, race and sex. These differing results may stem from Ford et al. (2010) looking at both iron and hemoglobin concentrations simultaneously with A1C status, which could potently offset the association.

5.2 Implications

The findings in this study provide additional understanding of the impact of ID on A1C levels. By conducting a propensity score analysis we determined the exposure effect of ID on odds of having an A1C ≥5.5%. Because ID is a common condition among women of child-bearing age, screening measures for diabetes should consider these findings when reporting A1C results to prevent erroneous results in iron deficient patients and even consider using other measures of assessing glucose control. For example, healthcare professionals could consider using direct measures of blood glucose level measurements, such as FPG and OGTT, for patients who are iron deficient. Research has been conducted to understand the mechanism in why ID

leads to increased A1C levels; however, there are no conclusive results. One hypothesis suggests that because A1C is a measure that represents the relationship between Hemoglobin A and serum glucose that is expressed as a percent of Hemoglobin A, a deceases in Hemoglobin A concentrations could potentially lead to an increase in A1C concentrations. (El-Agouza et al., 2002; Nathan, 2009).

5.3 Strengths and Limitation

The strengths of this research include the ability to declare exposure effect, data collected from a nationally representative population-based sampling frame, a large sample size, and standardization in measurement techniques. The application of propensity score analysis provided increased precision and a possible causal estimate of the association between ID and A1C. Utilizing NHANES's database and with the use of survey weights, this research is able to generalize its findings to all females of childbearing age in the US who meet the inclusion criteria in this analysis. Because NHANES is a national survey, it was able to provide this study with a large sample size providing a larger sample of ID women and therefore more stable group comparisons in our propensity score analysis. Different instrumentation was used in the measurement of A1C in NHANES, which may make combining these cycles questionable; however, the method of measuring A1C over a five-year period was tested and standardized so comparisons could be made.

There were also limitations in this study, one being the limited sample of women who had higher levels of A1C (>6.5%). Since women of childbearing age with diabetes were excluded from this analysis there were few who had A1Cs in the >6.5%, the traditional cutoff for a diabetes diagnosis. Therefore the women included in this study were primarily considered normal or pre-diabetic, limiting the generalizability of the findings to a higher cutoff.

Furthermore, this study used two propensity score methods, stratification of the propensity score estimate and utilizing the propensity score estimate as a continuous variable, Best practices suggest other approaches should also be considered, including matching and inverse probability weighting. Additionally, failure to include all relevant pre-exposure variables in the propensity model could limit the outcomes in this study. Lastly, while there is no correct way of addressing the complex survey elements within a propensity score analysis. The literature has different advice on which method is most appropriate. The decisions undertaken in this analysis uses an approach recommended by various authors (Zanutto (2006), DuGoff et all (2014)).

Another potential limitation was ensuring assumptions were met when developing the propensity score; this is critical to developing unbiased effect estimations. The positivity assumption can be assumed achieved looking at the overlapped histogram in Figure 1. If this assumption were not met, then there would not be enough information to compare ID status within the propensity scores to make accurate inference on the relationship with A1C levels. SUTVA seems reasonably met, as the ID status and A1C level in one woman is unlikely to impact ID status or A1C level for another woman. This is particularly true if these women were a simple random sample, it is possible with clustering that this assumption may not hold absolutely. Consistency and strong ignorability are both difficult to assess and may potentially lead to inaccurate propensity measures and invalid inferences. Because the consistency assumption looks at potential outcomes under counterfactual conditions that are not observed, it can only be assumed to be met. The strong ignorability assumption states conditional on a set of baseline covariates, ID status is independent from potential outcomes of A1C. While this assumption is untestable, it does require the propensity model to be properly specified, so that no other factors outside of the included covariates affect the probability of being ID. This leaves

room for error in determining the probability of ID due to the likelihood of unobserved covariates not being measured in an observational study is likely. These unmeasured covariates can affect the association between the independent and dependent variable if left unaccounted. Utilizing methods of propensity score analysis assists in estimating causals effects with only observed covariates. However, data used in this study is still observational and no statistical analysis can guarantee causal inference without a leap of faith that the assumptions are reasonably met and the approach is robust enough to allow some departure from the stated assumptions.

5.4 Conclusion

In summary, this study observed that ID is associated with A1C in multivariable regression analysis after controlling for covariates. This study also observed an effect between ID on A1C levels ≥5.5% of similar magnitude with the use of propensity score analysis, albeit slightly attenuated. Conducting a propensity score analysis generated balanced groups of women with and without iron deficiency. The result of this study suggests the need for awareness in the healthcare field for women within this population when assessing A1C results and guidelines for assessing iron deficient women's glucose levels may be needed, especially since women in this age group are considered to have a higher risk of being iron deficient. Future research should be conducted higher A1C levels to see if this effect holds true at levels greater than 6.5%.

Table 1. Weighted descriptive characteristics of 4656 women aged 12-49 years with and without iron deficiency, NHANES 2003–2008[1]

	Non-iron deficient	Iron deficient	
Variable	**(n=4122)**	**(n=534)**	**P-value[2]**
Age			
Mean (95%CI)	31.33 (30.87, 31.78)	32.86 (31.78, 33.94)	0.004
Income Class[3]			0.073
Lower Class	22.08	24.57	
Middle Class	29.11	30.83	
Upper Middle Class	15.31	18.42	
Upper Class	33.50	26.18	
Education			0.236
Less than High school	23.46	28.54	
High school graduate	19.59	21.77	
More than High school	56.95	49.69	
Race/Ethnicity			<.0001
Mexican American	9.26	13.51	
Non Hispanic White	68.15	52.80	
Non Hispanic Black	12.36	20.07	
Body mass index [4]			0.110
Under weight	4.68	2.38	
Normal weight	42.42	40.35	
Over weight	23.85	26.96	
Obese	29.05	30.31	
Mean (95%CI)	26.27 (25.84, 26.7)	26.61 (25.98, 27.26)	0.526
Poverty income ratio			
Mean (95%CI)	2.47 (2.36, 2.59)	2.09 (1.91, 2.28)	<.0001
Hemoglobin A1C (%)			
Mean (95%CI)	5.15 (5.13, 5.17)	5.31 (5.27, 5.35)	<.0001
Smoke			0.005
Yes	26.06	18.60	
No	73.94	81.40	
24-hour dietary recall of iron intake (mg)			
Mean (95%CI)	12.08 (11.8, 12.36)	11.2 (10.56 11.85)	0.003
Take Iron Supplements			0.001
Yes	31.28	20.63	
No	68.72	79.37	
Met Recommended daily allowance using iron supplements (≥18 mg/day)			0.001

Yes	21.09	12.09	
No	78.91	87.91	

[1] Age, BMI, and 24-hour dietary recall of iron intake are weighted arithmetic means (95% CIs); all other variables are expressed as weighted percentages.

[2] P-values comparing means or proportions are based either on the Wald F (means) Rao-Scott chi-square test (proportions)

[3] Income class is categorized into lower class ($0-$24,999), middle class ($25,000- $54,999), upper middle class ($55,000-$74,000), and upper class (>$75,000). Cutoffs chosen based of U.S Census Bureau from 2008 (DeNavas-Walt et al., 2009)

[4] Body Mass Index is categorized into Underweight (<18.5kg/m^2), Normal (18.5 – 24.9 kg/m^2), Overweight (25.0 – 29.9kg/m^2), and Obese (>29.9kg/m^2)

Table 2. Weighted multivariable survey logistic regression of A1C ≥5.5% and iron deficiency controlling for selected risk factors for women aged 12-49, NHANES 2003-2008

Variables				
	Beta est.	**Standard Error**	**P-value[1]**	**Weighted Adjusted Odds Ratio (95%CI)**
Body Iron				
ID	0.7421	0.1518	< .0001	2.100 (1.547, 2.851)
NID (Ref.)				Ref.
Age				
12-19	-1.4097	0.2217	< .0001	0.244 (0.156, 0.382)
20-39	-0.937	0.1153	< .0001	0.392 (0.311, 0.494)
40-49 (Ref.)				Ref.
Income[2]				
Lower Class	0.1376	0.1999	0.4947	1.148 (0.767, 1.716)
Middle Class	0.3851	0.1692	0.0275	1.470 (1.046, 2.066)
Upper middle class	0.2655	0.1921	0.1737	1.304 (0.886, 1.920)
Upper class (Ref.)				Ref.
Education				
Less than High school	0.2677	0.186	0.1568	1.307 (0.899, 1.900)
High School Diploma (including GED)	0.1994	0.1733	0.2559	1.221 (0.861,1.730)
More than High school (Ref.)				Ref.
Race				
Mexican American	0.5511	0.1945	0.0068	1.735 (1.173, 2.567)
NH Black	0.911	0.2043	< .0001	2.487 (1.649, 3.752)
NH white (Ref.)				Ref.
Smoke				
Yes	-0.0117	0.1128	0.9177	0.988 (0.788, 1.240)
No (Ref.)				Ref.
Met Recommended daily allowance using iron supplements				
No	0.1157	0.1903	0.5461	1.123 (0.765, 1.647)
Yes (Ref.)				Ref.
24-hour dietary recall of iron intake (mg)				
	0.00104	0.00891	0.9078	1.001 (0.983,1.019)
BMI (kg/m^2)				
	0.0934	0.00977	< .0001	1.098 (1.077, 1.120)

[1] P-values based Wald Chi-square.

[2] Income class is categorized into lower class ($0-$24,999), middle class ($25,000- $54,999), upper middle class ($55,000-$74,000), and upper class (>$75,000). Cutoffs chosen based of U.S Census Bureau from 2008 (DeNavas-Walt et al., 2009)

Table 3. Proportion of A1C ≥5.5% among women aged 12-49 participating in NHANES cycles 2003-2008 who had ID or NID according to percentiles of propensity score

Iron deficient				
Percentile	**Mean Propensity Score**	**n**	**Frequency A1C ≥5.5%**	**Percent A1C ≥5.5%**
80 to 100	0.1818	144	59	41.0
60 to <80	0.1289	80	27	33.7
40 to <60	0.1104	87	20	23.0
20 to <40	0.0864	98	23	23.5
0 to <20	0.0584	34	4	11.8
Overall	0.1276	443	133	30.0

Non-iron deficient				
Percentile	**Mean Propensity Score**	**n**	**Frequency A1C ≥5.5%**	**Percent A1C ≥5.5%**
80 to 100	0.1758	641	198	30.9
60 to <80	0.1287	607	91	15.0
40 to <60	0.1107	706	119	16.9
20 to <40	0.0852	991	118	11.9
0 to <20	0.0553	598	62	10.4
Overall	0.1091	3543	588	16.6

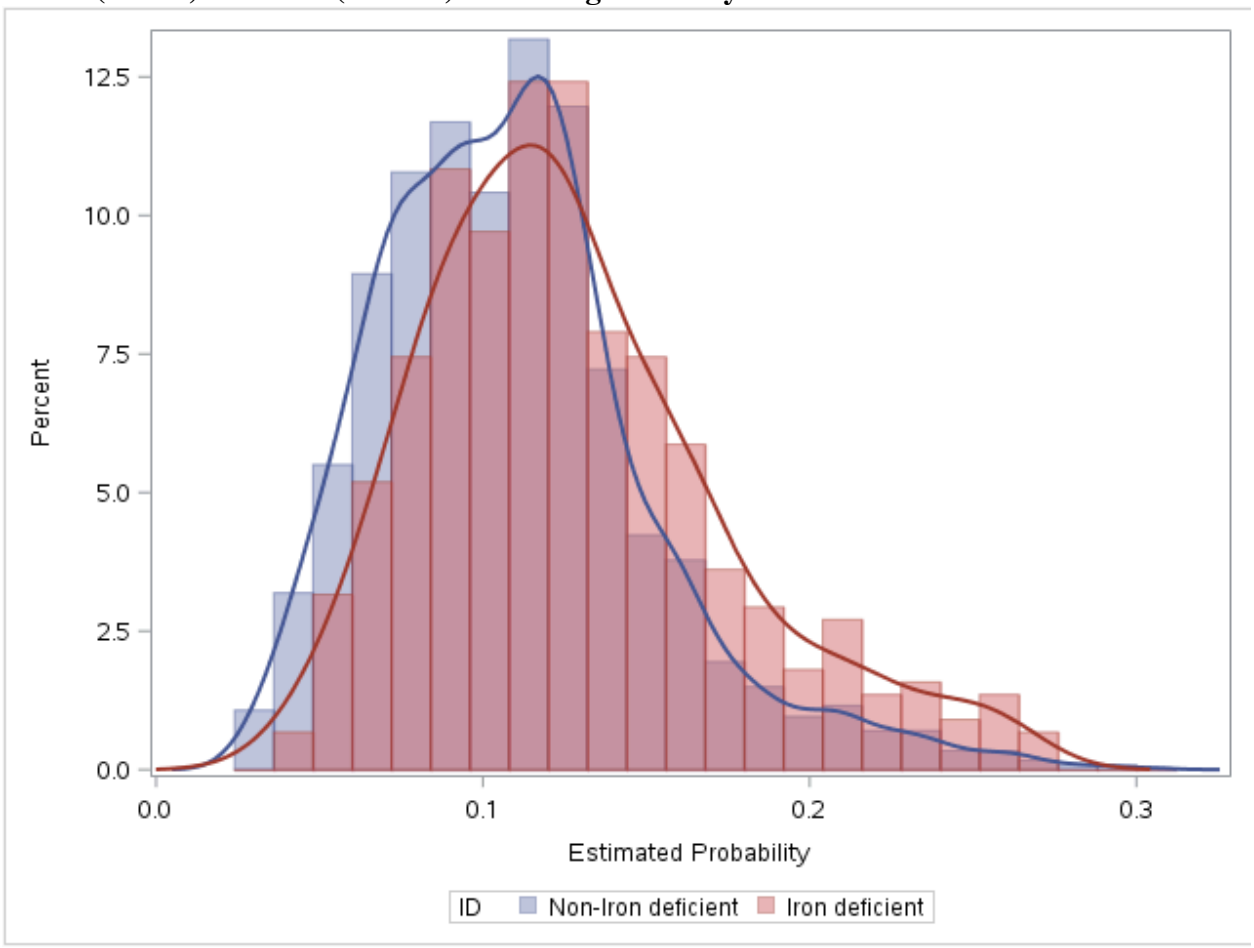
Table 4. Comparison of the estimated odds ratio between ID and A1C(≥5.5%) using survey logistic regression, and propensity score analyses for women aged 12-49, NHANES 2003-2008

Table 4			
Models	**N**	**OR[1]**	**95% CI**
Crude Model	4656	2.32	1.75, 3.07
Multivariable Model[2]	4634	2.10	1.55, 2.85
Propensity score (Continuous)	3986	1.88	1.41, 2.49
Propensity score (Quintiles)	3986	1.92	1.45, 2.55

[1] Weighted odds ratio

[2] Adjusted by age, income, education level, race, BMI, smoking status, 24-hour dietary recall of iron intake, Met Recommended daily allowance using iron supplements RDA

Figure 1. Histograms and estimated probability density functions of the propensity scores for ID (n=443) and NID (n=3543) women aged 12-49 years old.



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