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Hua Fang
University of Nebraska-Lincoln

Craig Johnson
University of Nebraska-Lincoln

Christian Stopp
University of Nebraska-Lincoln

Kimberly Andrews Epsy
University of Nebraska-Lincoln, kespy2@unl.edu

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A New Look at Quantifying Tobacco Exposure during Pregnancy Using Fuzzy Clustering

Hua Fang, **Craig Johnson**, **Christian Stopp**, and **Kimberly Andrews Espy**[*]

Department of Psychology & Office of Research University of Nebraska-Lincoln Lincoln, Nebraska, USA, 68588-0433

Abstract

Background—Prenatal tobacco exposure is a risk factor for the development of externalizing behaviors and is associated with several adverse health outcomes. Because pregnancy smoking is a complex behavior with both daily fluctuations and changes over the course of pregnancy, quantifying tobacco exposure is a significant challenge. To better measure the degree of tobacco exposure, costly biological specimens and repeated self-report measures of smoking typically are collected throughout pregnancy. With such designs, there are multiple, and substantially correlated, indices that can be integrated via new statistical methods to identify patterns of prenatal exposure.

Method—A multiple-imputation-based fuzzy clustering technique was designed to characterize topography of prenatal exposure. This method leveraged all repeatedly measured maternal smoking variables in our sample data, including (a) cigarette brand; (b) Fagerstrom nicotine dependence item scores; (c) self-reported smoking; and (d) cotinine level in maternal urine and infant meconium samples. Identified exposure groups then were confirmed using a suite of clustering validation indices based on multiple imputed datasets. The classifications were validated against irritable reactivity in the first month of life and birth weight of 361 neonates (Male$_n$ = 185; Female$_n$ = 176; Gestational Age$_{Mean}$ = 39 weeks).

Results—This proposed approach identified three exposure groups, non-exposed, lighter-tobacco-exposed, and heavier-tobacco-exposed based on high-dimensional attributes. Unlike cutoff score derived groups, these groupings reflect complex smoking behavior and individual variation of nicotine metabolism across pregnancy. The identified groups predicted differences in birth weight and in the pattern of change in neonatal irritable reactivity, as well as resulted in increased predictive power. Multiple-imputation based fuzzy clustering appears to be a useful method to categorize patterns of exposure and their impact on outcomes.

Keywords

Prenatal tobacco exposure; fuzzy clustering; multiple imputation; exposure pattern; irritable reactivity

[*]**Corresponding Author:** Kimberly Andrews Espy Office of Research, University of Nebraska-Lincoln 303 Canfield Administration Building Lincoln, NE, USA, 68588-0433 Ph: 402.472.2851, Fax: 402.472.3834 kespy2@unl.edu.

6. Conflict of Interest

The authors have declared no conflicts of interests related to this research.

1. Introduction

Approximately 20% of U.S. women smoke during pregnancy resulting in at least 500,000 prenatally exposed newborns [3]. Prenatal tobacco exposure (PTE) is associated with an increased risk of externalizing behavior, psychiatric disorders (e.g., [17,34,37,50,70,71]), and other adverse physical health outcomes, including *in utero* growth restriction, prematurity, low birth weight, and pediatric asthma and ear infections [20,31,67]. One challenge in characterizing the impact of PTE is quantifying exposure. Pregnancy smoking is a complex behavior, typically with substantive daily variation [52], and substantial within person variation over the course of pregnancy [51]. Equally as complex is nicotine metabolism that differs among women and across pre-, during-, and post-pregnancy periods [7,8,18,40]. Because pregnant smokers vary with respect to their smoking behavior, nicotine dependence, and metabolism, intuitively we can assume that there are clusters of smokers who can be defined by these attributes. For example, one woman may be a heavy smoker with more than ten cigarettes per day with a quick nicotine metabolism but her frequency of smoking and nicotine dependence may be steadily declining because of successful quitting. Another woman may be a light smoker, persisting across pregnancy, and yet with a slower nicotine metabolism. Therefore, in many studies, the type of smoker often is defined by the particular variable of interest (lighter vs. heavier nicotine dependence, quick vs. slow nicotine metabolism, high vs. low frequency of smoking, persistent smoker vs. quitting or slowly declining smoker). Thus, the full topography of tobacco exposure is more complex than the "exposed" and "non-exposed" groupings conventionally determined by an inflexible, predetermined cut-off score on self-reported smoking measures or biological assays.

Traditionally, self-reports of smoking behavior, or repeated biological specimen assays, have been used to characterize exposure, each with its own advantages and disadvantages. Self-report measures capture smoking amounts over time; biospecimens provide an objective measurement of exposure that can reduce under reporting biases [22]. Self-reported smoking, however, is affected by recall bias and under-reporting [25] while bio-assays only reflect recent smoking and are influenced by metabolic variations. To deal with these limitations many researchers define exposure groups based on self-reported exposure (e.g., [17,34,37,50,53,71]) and use biospecimen data to validate group assignment [26].

With the advancement of statistical methodologies, researchers are exploring new methods to improve group classification. For instance, Dukic et al. [22,23] statistically adjusted self-reported measures with available bioassay data to calibrate self-reported smoking to include metabolic differences reflected in the cotinine values derived from the biospecimen samples. Although this method requires deriving underlying statistical distributions, set quantity thresholds and other constraints, it illustrates that both self-report measures and biological assays contain unique information about exposure that can be used together. By using this method, Dukic et al. were able to account for report bias commonly found in self-report measures. To date, multiple sources of data only have been used to calibrate self-reported number of cigarettes, but have not been fully leveraged to define empirically the topography of exposure.

An alternative to define the topography of exposure is to utilize all available smoking data sources [4]. In the statistical literature, multiple sources of related information are called high-dimensional attributes. Using high-dimensional attributes to empirically define clusters of pregnant smokers should better account for individual variability and result in better characterization of the topography of exposure. However, this strategy poses substantial modeling and computational challenges. Among available techniques, fuzzy clustering methods are most appropriate for handling high-dimensionality in smoking data. These

fuzzy methods better accommodate actual smoking behavior which is more continuous in nature [46,51], by allowing individuals to be members of multiple clusters with degrees of membership [10,12]. The fuzzy clustering method is in contrast to conventional clustering models, such as hierarchical clustering and K-means hard clustering, which only allow an individual to belong to a single cluster [12]. For example, a participant with a cotinine value of 1119 ng/mL has to be assigned to the exposed or non-exposed group, even though the cotinine might be on the borderline if a cut-off value of 1200 ng/mL is used. In practice, pregnant smokers can be a member of multiple clusters to varying degrees across pregnancy depending on their smoking behavior and when the measurements are taken. For example, a smoker may have a higher degree of membership in a "lighter-smoker" cluster and a lower degree in a "heavier-smoker" cluster due to fluctuations in her smoking during pregnancy. Therefore, fuzzy clustering is more useful in time-varying situations where cluster membership can overlap. Fuzzy clustering methods also enhance cost-effectiveness by enabling the use of all available exposure measures, which are costly and time consuming to collect, but often end up ignored in the final analyses.

The purpose of this study was to examine whether including all exposure related measures into a fuzzy clustering model would result in better classification of the topography of prenatal tobacco exposure. We then validated our results by examining the effect of the new exposure grouping on irritable reactivity (IR) in neonates, measured by regulatory responses to auditory and visual stimuli, as well as to routine handing [13,32,38,44,49,51,68]. We hypothesized that fuzzy clustering would improve the characterization of the impact of exposure on this outcome by refining exposure measurement through identifying neonates with similar exposure patterns, and thus conserving power. We then used birth weight, the most commonly reported outcome that is affected deleteriously by prenatal tobacco exposure [20,21,41,48,66,72], to cross-validate the utility of the fuzzy clustering methods. We hypothesized those identified as heavier smokers would have neonates of lower birth weight compared to those born to non-smokers.

2. Methods

2.1. Participants

Data from the Midwest Infant Development Study (National Institutes of Health R01 DA014661; Espy, PI), a project designed to assess the impact of prenatal tobacco exposure on neonatal regulatory skills, was used. Detailed recruitment and enrollment procedures for this study are provided in Espy et al. [26], and more sample characteristics are provided in Fang et al. [28]. Briefly, pregnant mothers responded to flyers distributed at two sites in the Midwest: a rural tri-county region and a small city. Interested mothers phoned the laboratory, where trained screeners gathered demographic information and determined study eligibility. Mothers were eligible if they planned to deliver in a local hospital; spoke English; drink no more than four drinks per day; and did not use illegal drugs. All smoking pregnant women who were actively smoking during pregnancy or reported smoking around the last menstrual period (LMP, [25]) were enrolled, with 46% of smokers reported smoking 10 or more cigarettes per day prior to pregnancy. Eligible non-smokers were oversampled for enrollment based on Medicaid insurance status (a less intrusive proxy for income), race/ethnicity, and education (<14 years) to render exposure groups more comparable on variables that are related to smoking and to child outcomes. In spite of our efforts to eliminate illegal drug users at screening, 53 women admitted use of marijuana during subsequent prenatal interviews or their child tested positive for marijuana at birth. We retained this data to capture heavier smokers who are also more likely to use marijuana during pregnancy. However, data from eight participants with heavy drinking during any prenatal month (> 1 drink/day), one participant who was prescribed anti-psychotic medication throughout pregnancy, and 17 participants who were born < 35 weeks gestation

were excluded because of the known, large effects associated with these variables on neonatal outcomes.

The final sample included 361 full term neonates (176 females, 185 males). According to traditional methods of using maternal self reports and confirmation by biospecimen samples [26], 189 were initially assigned as tobacco-exposed (TE) and 172 as non-exposed (NE). Within each exposure group, males and females were approximately equal in frequency (χ^2 (1, $N_{NE} = 172$) = .023, $p =0.879$; χ^2 (1, $N_{TE} = 185$) = .259, $p = 0.636$). The race/ethnicity of the majority of pregnant women was White, non-Hispanic (77%), with no difference between TE and NE groups in racial/ethnic composition ($TE_{white\_n} = 144$, $TE_{African\_n} = 25$, $TE_{Hispanic\_n} =13$, $TE_{native\_n} = 5$, $TE_{Asian\_n} = 1$, $TE_{other\_n} = 1$, $NE_{white\_n} = 133$, $NE_{African\_n} = 23$, $NE_{Hispanic\_n} =11$, $NE_{native\_n} = 3$, $NE_{Asian\_n} = 2$, $NE_{other\_n} = 0$, χ^2 (5, $N = 361$) = 1.723, $p > 0.80$). Exposure groups also were comparable in socio-economic background (represented by Medicaid insurance, $TE_{Medicaid\_\%} = 85$, $NE_{Medicaid\_\%} = 84$, $p > .80$) and monthly family income, ($TE_{median\_\$} = 1450$, $NE_{median\_\$} =1730$, $p > .19$). As is the case in many observational studies [59-61], smoking and non-smoking women differed on a variety of background variables including alcohol use in the first trimester ($TE_{average\_drinks\_perday} = 0.12$, $NE_{average\_drinks\_perday} =0.02$, $p < .001$), age at delivery ($TE_{at\ delivery} = 25.2$, $NE_{at\ delivery} = 26.6$, $p < 0.01$), marital status ($TE_{married\_\%} = 37$, $NE_{married\_\%} = 57$, $p < .001$), education level ($TE_{years} = 12.98$, $NE_{years} = 13.88$, $p < .001$), prenatal weight gain ($TE_{pounds} = 35.5$, $TE_{pounds} = 29.4$, $p < 0.01$), depression symptoms ($TE_{BSI\_Tscore} = 53.70$, $NE_{BSI\_Tscore} = 51.21$, $p < 0.01$), anxiety symptoms ($TE_{BSI\_Tscore} = 50.70$, $NE_{BSI\_Tscore} = 48.59$, $p < 0.05$), and prescription medications during pregnancy (e.g., $TE_{Thyroid\_\%} = 2$, $NE_{Thyroid\_\%} = 4$, $p < 0.05$) [28]. To minimize the selection bias resulting from background differences, we used previously estimated propensity scores [28] derived from more than 40 confounding variables of background demographics, diet, weight, exercise habits, other prenatal substance use, prescription medication, and from the resultant standardized scaled scores from the Brief Symptom Inventory [19], Conners ADHD Rating Scale (Short) [16], and the Woodcock-Johnson Brief Intellectual Ability assessment [73]. Propensity scores were calculated using non-parametric generalized boosted models which handle non-linearity, interactions among variables, and ignorable missing values (e.g., [42,47,59]).

2.2. Self-reported measures, biospecimen and irritability reactivity

Enrolled pregnant women completed structured interviews at 16-weeks, 28-weeks, and just after delivery (termed 40-weeks) using standard, timeline follow-back methods regarding their smoking behavior. During each interview, mothers were asked the average number of cigarettes they smoked per month since their last visit. In addition, participants provided preferred brand, inhalation patterns, and items from the revised Fagerstrom Test (FTND, [33]) for nicotine dependence at each interview. Participants also provided urine samples at each interview from which the maternal cotinine levels were derived by the DRI® Cotinine from US Drug Laboratories. Neonates' cotinine levels were measured from a meconium sample taken from the infant's diaper shortly after birth (DRI® Cotinine Assay from US Drug Laboratories).

Irritability reactivity [26] was derived empirically using principal axis factor analysis from items administered as a part of the Neonatal Temperament Assessment (NTA), a standardized assessment with demonstrated reliability (0.72-0.99) and predictive validity [26,45,55-58]. The NTA was administered shortly after birth, and at 2- and 4- weeks of age and consists of four modules: Attention/Orientation, Cold Disc Stressor, Pacifier Withdrawal, and Soothing Maneuvers. The four modules are administered in a fixed sequence between feedings to capitalize state variation as a function of the neonates' natural sleep-wake cycle. The IR factor score is composed of seven items that were scored during the administration of the Attention/Orientation module where the neonate's reaction to

auditory and visual stimuli, as well as reflex maneuvers, was scored. Auditory stimuli included a bell, rattle, and the examiner's voice, which were presented on the right and left side three times for a total of 18 trials. Visual stimuli included a bulls-eye and the examiner's face, where each stimulus was positioned first at the center of the visual field, moved around the neonate's head to the right or left at a 90° angle, back to the center, around the other side at a 90° angle, and then back to the center for 4 trials.

Examiners also administered ocular reflexes, optic blinks, acoustic blinks, and rotation and the elicitation of rooting, sucking, withdrawal to toothpick prick, and Moro reflexes. After attention/orientation behaviors were scored, the neonate's latency to cry/soothe (in seconds) and the degree of irritability during these maneuvers (1 = not irritable; 5 = highly irritable) was scored. Examiners also provided summary ratings of the neonate's reinforcement value throughout the module (1 = glad to be finished; 5 = fun to have at home). In this study, the seven IR item scores was retained as the dependent measure to index irritable reactivity at birth, 2-, and 4- weeks of age, respectively. The second dependent measure for cross-validation purposes was the baby's birth weight (in grams) recorded by hospital staff at delivery.

2.3. Fuzzy clustering procedures

2.3.1 Step 1: Variable selection—
Variables in the fuzzy clustering model were selected to maximize information relevant to exposure based on extant literature. Twenty-two variables among four categories of exposure information were collected, including biospecimen assayed cotinine from maternal urines and neonatal meconium; Federal Trade Commission [1] nicotine levels in identified preferred cigarette brand; number of self-reported cigarettes per day; and dependence as measured by the FTND [33]. To measure consumption across pregnancy, the average self-reported number of cigarettes smoked per day for each month during pregnancy (9 variables) was used. Assayed cotinine levels in maternal urine samples collected at the three prenatal interviews (3 variables) and in neonatal meconium collected shortly after birth (1 variable) were selected to reflect variability in both the amount of smoking and maternal nicotine metabolism, as well as in exposure to environmental tobacco smoke. The amount of nicotine in the preferred brand of cigarette reported by mother at each interview was included to index nicotine potency (3 variables). Finally, the average FTND item scores across 16, 28, and 40 weeks (6 variables) was included to represent nicotine dependence [33]. Table 1 presents descriptive statistics for these variables.

2.3.2 Step 2: s-FCM modeling—
Although FCM has been shown to be a valid and computationally efficient clustering method, it cannot use datasets with missing values [10]. Missing data is an inevitable characteristic in longitudinal studies due to attrition, dropout, and other methodological issues [27,39]. In this sample, 9 out of 22 variables had missing values, ranging from 0.6% to 18.3% of the observations. To account for missing data, we designed a multiple-imputation-based Fuzzy c-Means Model (s-FCM). s-FCM incorporates multiple imputation techniques during the clustering procedure. Specifically, s-FCM estimates missing values a specified number of times. Because three to five imputations were adequate in multiple imputation [62], for this study, we imputed missing values five times and generated five complete datasets. Clusters were then identified in each of the five imputed datasets. Next, an *exposure clustering inconsistency rate* was calculated to test the sensitivity of s-FCM for its robustness to missing values, where the larger the rate, the less stable the algorithm. Because we knew the classification of non-smokers and smokers with near certainty but not whether there are different clusters within pregnant smokers, an *exposure clustering accuracy rate* was evaluated by comparing s-FCM derived cluster labels to binary "smoker" vs. "non-smoker" groupings derived traditionally by self-reported

smoking with confirmation by biospecimen results. Finally, to highlight differences, s-FCM generated clusters were compared with those derived from typical clustering methods that do not permit fuzzy membership. We compared s-FCM with hierarchical clustering [43] and K-means hard clustering using *exposure clustering inconsistency* and *accuracy rates.* A more detailed explanation of the statistical specifications for s-FCM modeling is provided in the Appendix.

2.3.3 Step 3: s-FCM Cluster Validation—The number of clusters was identified using multiple-imputation-based fuzzy clustering indices; graphics and statistical testing; and subsets of exposure variables. This multiple validation procedure provides a comprehensive assessment of the performance and stability of the s-FCM, which is more effective than typical single clustering index-based validation. The following sections describe these procedures.

<u>**2.3.3.1 s-FCM Indices:**</u> Xie and Beni's index (XB) [74] is a widely used index for fuzzy clustering that includes both a geometric and statistical approach [12], because it quantifies the ratio of the total variation within clusters and the separation of clusters. The smallest value of XB indicates the optimal number of clusters. The multiple-imputation-based XB_m in our study was modified to comply with our multiple imputed data sets (see Appendix).

Four other validation indices [9,11] were modified for multiple imputation data. These included: (a) Partition Coefficient (PC_m, smaller = better); (b) Partition Entropy (PE_m, larger = better); (c) Partition Index (PI_m, smaller = better); and (d) Separation Index (SI_m, larger = better). The main drawback of PC and PE are their monotonicity (decreasing or increasing) with the number of clusters and lack of direct connection to data, while PI and SI are more useful in comparing algorithms [12]. Because of the known individual weaknesses, we considered all indices for validation.

<u>**2.3.3.2 Graphics and Statistical testing:**</u> To visualize the cluster results from high dimensional attributes in two-dimensional space, Sammon mapping [6,63] was used. For each potential cluster, functional curves for each exposure-related repeated measure were displayed to examine the number of valid robust clusters. We then statistically tested if the identified clusters differed on included exposure attributes, to validate empirically the obtained exposure clusters.

<u>**2.3.3.3 Subsets of tested exposure variables:**</u> Subsets of the included attributes were examined to determine if redundancy existed in the s-FCM models using the exposure clustering accuracy rate. We removed one subset of repeatedly-measured variables, calculated exposure clustering accuracy rate, placed it back into the model, and repeated the process. If the *exposure clustering accuracy rates* decreased using a subset, the original variables were retained; if the accuracy rates did not change, then the subset was used to determine exposure topography. This strategy maximized the information used to classify exposure at minimal model complexity.

2.4. Testing predictive power of identified exposure groups

To evaluate the predictive power of s-FCM identified groups, two sets of analyses were conducted. First, the s-FCM-identified exposure groups (i.e., latent patterns/clusters) were used to examine the impact of prenatal tobacco exposure on the pattern of change in irritability reactivity across the neonatal period. In previous work from this dataset using the traditionally defined binary exposure variable (0 = NE, 1 = TE), neonates displayed observable, but non-significant, differences across the neonatal period in irritable reactivity, [26]. Consistent with Fang et al. [28], non-exposed (NE) were set as the base group in both

analyses, and dummy variables used to compare s-FCM derived groups to NE in the same multiple indicator growth model (MIGM) with propensity score covariates. Our aim was to determine whether the s-FCM methods would reveal a more nuanced picture of the impact of exposure on the pattern of change in IR scores across the neonatal period, for example, whether those who were exposed more heavily might show unique vulnerability. Second, a cross-validation test was performed on an outcome that has been indisputably associated with deleterious outcome, birth weight [21,24,41,48,66,72] to confirm the efficacy of our proposed technique, where the s-FCM identified exposure groups were used to predict birth weight. To increase modeling precision in both validation analyses, propensity scores were used. Propensity scores were estimated [28] from more than 40 confounding variables covering background demographics, diet, weight, exercise habits, other prenatal substance use, prescription medication, and from the standardized scaled scores from the Brief Symptom Inventory [19], Conners ADHD Rating Scale: Short [16], and the Woodcock-Johnson Brief Intellectual Ability assessment [73].

3. Results

Across five imputed datasets, s-FCM resulted in a 0% inconsistency rate, while hierarchical clustering and K-means hard clustering yielded a 50% and 20% rate of inconsistency, respectively. The clustering accuracy rate of s-FCM was 100%, with the accuracy rates for the K-means of 97%, and Hierarchical clustering of 48%. Although the K-means clustering approach was adequate, the s-FCM showed the best classification.

3.1 Fuzzy Clustering Indices

As shown in Figure 1, three optimal clusters were revealed by the minimum value of XB_m. The other four validation indices (PC_m, PE_m, PI_m, SI_m) also pointed to three clusters, although the weakness (e.g., monotonicity) of PC_m and PE_m showed minimal difference or trivial advantage at larger number of clusters in comparison to three clusters.

3.2 Graphics and Statistical testing

Sammon mapping (Figure 2) further supported three clusters, where asterisks represent the projected centroids and dots represent subjects within the identified clusters. The values on the two axes are the projected normalized scores for these subjects. Furthermore, Figure 3 displays two sets of functional curves of these three potential clusters for our selected repeated measures: urine cotinine levels (lower panel) and self-reported cigarettes per day (upper panel) for each month during pregnancy. These visual results further reinforce the quantitative results that indicate two clusters exist within pregnant smokers.

Table 1 displays significance levels of the differences between two identified smoker clusters (heavier-Tobacco-Exposed: hTE; lighter-Tobacco-Exposed: lTE) on the included maternal smoking variables. Although the hTE and lTE groups differed in the number of previous pregnancies, first-trimester exercise, and one psychopathology scale, the groups were comparable on most background variables (see Table 2). Among the neonates born to women n the two identified clusters of the pregnant smokers, 40 were hTE and 149 lTE, with 172 neonates in the non-exposed (NE) comparison group.

From the decriptive statistics and graphs generated from s-FCM (Table 1, Table 2, and Figure 3), a gradient of lighter and heavier smokers were identified. These decriptives present actual individual variation of cotinine levels in conjunction with their self-reported smoking patterns during pregnancy, nicotine dependence and consumption. Although heavier and lighter smoking groups differed on variables shown in Table 1, the two groups did not differ on many of the background variables (Table 2) that routinely are reported to

differ between smokers and non-smokers. This pattern of differences between heavier and lighter exposure groups reinforces that clustering women based on routine background variables likely would not be useful to uncover meaningful sub-groups of exposure topography.

3.3 Subsets of tested exposure variables

To evaluate attribute redundancy, we tested five subsets of the original variables: nicotine in cigarette brands, FTND scores, self-reported number of cigarettes during pregnancy, urine and meconium cotinine. Exposure clustering accuracy rates dropped from 100% to 93%, 90%, 64%, 60% and 50%, respectively. This means that 27, 36, 129, 144, and 181 of the 361 mothers were misclassified when variables were dropped from the model. The difference in accuracy rates occur because different sets of variables resulted in different cluster centroids (equivalent to means) and Euclidean distance (equivalent to variance). Subset analyses indicated that none of subsets were redundant and all provided important information. Our results suggest that including all our selected variables provided valuable information used to develop the exposure clusters and all variables were necessary for accurate clustering.

3.4 Testing predictive power of identified exposure groups

Using the latent multiple indicator quadratic growth model and propensity score covariate to model confounding influences from Fang et al. [28] (shown conceptually in Figure 4), centered at age of four weeks, the two-fuzzy-cluster-derived exposure-group indicators (lTE and hTE, with NE as comparison group) predicted the intercepts, linear slope and quadratic acceleration of IR ($IR_{i,s,q}$). In this model, we also included birth gestational age (in weeks), sex (Male =1 and Female = 0), and the interaction of sex and exposure group. Compared to NE neonates, those who were hTE had significantly higher IR scores and a faster linear slope on average at 4 weeks of age ($\gamma_{2\_i*hTE} = 0.239$, SE = 0.069, $p = 0.001$; $\gamma_{2\_s*hTE} = 0.183$, SE = 0.097, $p = 0.060$), as well as a marginally higher rate of acceleration ($\gamma_{2\_q*hTE} = 0.034$, SE= 0.022, $p = 0.118$). In contrast, lTE and NE neonates did not differ in the pattern of IR change. Moreover, the impact of hTE on IR growth parameters significantly differed between boys and girls ($\gamma_{6\_i*hTEbySex} = -0.243$, SE = 0.093, $p = 0.009$; $\gamma_{6\_s*hTEbySex} = -0.263$, SE = 0.114, $p = 0.021$; $\gamma_{6\_q*hTEbySex} = -0.054$, SE = 0.026, $p = 0.035$). hTE females were more irritable than hTE males and NE neonates at four weeks of age, and also IR scores changed with greater linear slope and quadratic acceleration (see Figure 5). In comparison to the models in Fang et al. [28] where exposure groups were defined conventionally by self-reported smoking with confirmation by biospecimen sample results, the average R^2 for predicting each growth parameter was 13% higher using the s-FCM techniques. This difference indicates a substantive gain in predictive power resulted from using clusters identified with the s-FCM model in comparison to traditional binary grouping methods.

Turning to birth weight, the s-FCM showed that hTE neonates weighed significantly less at birth than their NE peers ($\gamma_{\_hTE/NE\_bwt} = -218.62$, $p = 0.036$), but the lTE neonates did not ($\gamma_{\_lTE/NE\_bwt} = -100.87$, $p = 0.249$). The estimate of exposure group effects on birth weight from using the traditionally defined binary exposure grouping was $\gamma_{\_TE/NE\_bwt} = -131.96$, $p = 0.121$. The difference in R^2 using the s-FCM method was large, an increase of 36%, demonstrating the substantial precision gained with the s-FCM approach.

4. Discussion

The purpose of this study was to examine the utility of a new method to define the topography of tobacco exposure across pregnancy by recognizing the changes in smoking behavior during pregnancy and modeling exposure group membership as more than a single,

selected cut-off score. Fuzzy clustering statistically enables better characterization of like groups by leveraging the continuous nature of behaviors/measures and utilizing the complexity of actual individual values on high dimensional attributes. The impact of this new technique is evident within this dataset. Many women report terminating smoking before the second trimester, but biospecimen results were not always consistent with self-reports. In this sample, 69 (37%) women reported quitting prior to the second trimester, and yet sample mean cotinine values from 16- and 28-week biospecimens did not differ [26]. Furthermore, some women did not smoke daily and discriminating the differential effects of the duration of smoking versus the amount of smoking is difficult in humans. The results from s-FCM modeling suggest that women who smoke more cigarettes, do so throughout pregnancy, and prefer brands containing more nicotine, can be empirically discriminated from those who smoke less and may or may not successfully quit during pregnancy. This new fuzzy clustering approach provides systematically quantified information on the topography of exposure rather than the typically used, traditionally defined binary cutoff-score based (exposed, non-exposed) group assignment.

In addition to its ability to capture and empirically model variation related to complex smoking behavior, s-FCM enables use of all gathered exposure data to classify exposure levels rather than relying upon a single, or a small number of, measures. In this study, four categories of exposure data that included biological and self-report measures were used; in total, 22 measurements determined exposure groups. The ability to include all data undoubtedly helps compensate for the weakness of any individual measure or method.

Our proposed s-FCM is also the first FCM model designed for use in longitudinal research where missing data is prevalent. In s-FCM, missing data is estimated using multiple-imputations, which in turn generate multiple datasets that were examined for consistency of classification. The proposed s-FCM then uses a multi-method approach to group classification. Specifically, s-FCM calculates a suite of cluster validation indices based on the multiple imputed data sets to help empirically identify the optimal number of clusters. These results were then tested statistically and visually displayed using Sammon mapping. To maximize available data but use the most parsimonious model, s-FCM tests for attribute redundancy by eliminating a set of repeated measures and then reexamining the exposure clustering accuracy rate.

The predictive power of s-FCM identified of two latent sub-groups of smokers (lighter vs. heavier) was demonstrated on both a behavioral and a biological outcome. Our results for neonatal irritable reactivity revealed that females in the heavier exposed group had the greatest risk for sustained differences and persistent elevations in irritable reactivity at four weeks of age. This finding is consistent with the emerging picture of a heightened vulnerability of females to tobacco exposure also observed at adolescence [69]. Given the importance of irritability as a signal to elicit care giving, these early differences in regulatory skills likely set the stage for the ensuing deviations in maternal-infant behavior that have been observed by others [64,65]. These differences may be an early precursor of later deviations in emotional dysregulatory behavior [15,70] and eventually in clinical symptomatology [34,37,50,71]. Of course generally, neonatal abilities have not been shown to be strong predictors of later outcomes [14], but improved psychometrics and new neonatal instruments show promise [36].

s-FCM identified neonates who incurred heavier tobacco exposure showed a faster rate of increase in irritable reactivity compared to non-exposed peers during the first month of life. Because no differences in the pattern of change in irritable reactivity was noted between lighter-exposed and non-exposed neonates, our results suggest that only a subgroup of those exposed may be at risk for heightened irritability in reaction to routine handling and

stimulation. In our previous results [26,28] differences in the pattern of change in irritable reactivity were non-significant using the traditionally derived, binary exposure group classification. This study's findings help to clarify the mixed extant literature regarding neonatal irritability and prenatal tobacco exposure [13,29,35,54]. Specifically, only those who are more heavily and persistently exposed show alterations in the pattern of change in irritability.

Cross validating s-FCM groups using birth weight as a criterion, revealed heavier exposed neonates also weighed less at birth than their non-exposed peers, while lighter exposed neonates did not. This finding reinforced the validity of our s-FCM, as the impact of prenatal tobacco exposure on birth weight is indisputable [20,21,41,48,66,72]. Interestingly, our sampling strategy for prospective recruitment included comparable ascertainment of women who smoked 10 or more cigarettes/day around conception with the goal to yield adequate numbers of heavier smokers. Prospective recruiting during pregnancy makes it impossible to control the resultant pattern of smoking during pregnancy (because the smoking occurs after enrollment). Our results suggest that using such ascertainment criteria may not be effective, given the much smaller number of women who were classified in the heavier group based on the multiple indices of prenatal smoking. Taken as a whole, s-FCM modeling provides a new and exciting way to empirically define groups based on multiple measures collected repeatedly. With s-FCM, all available smoking data were leveraged, missing values accommodated, and the predicative power of models was increased.

Although s-FCM has obvious methodological advantages, special concerns regarding the generalization of this method need to be addressed. First, it is important to note that variable selection is an important step in s-FCM procedure. In this study, we used 22 variables to characterize tobacco exposure topography. However, the designs of other studies likely differ in the number and type of smoking variables, as well as in the sampling frequency. Based on our s-FCM findings, repeated measures of (1) self-reported cigarettes, (2) cotinine levels, (3) nicotine dependence scores, and (4) amount of nicotine contained in cigarettes of preferred bands were critical attributes. Therefore, we suggest including monthly, or at least trimester, variables of self-reported number of cigarettes per day; trimester sampling of maternal biospecimens; and neonate meconium sampling. Although all of the FTND item scores may not be used in all studies, but to our knowledge, most include similar questions regarding nicotine dependence, such as "how many cigarettes per day do you smoke" and "how soon after you wake up do you smoke your first cigarette?" These variables played an important role in exposure characterization and added to group classification. Furthermore, the preferred cigarette brand data are commonly gathered and it is possible to estimate the amount of nicotine contained in brands. In short, we recommend including all available information in the sample dataset, as the s-FCM optimization procedures empirically determine the most effective information subset for classification. This proposed exposure variable selection strategy reflects the nature of our cost-effective s-FCM approach, that is, with this method, researchers are not forced to use only a single exposure variable, when time and money were spent to collect multiple measures. Rather, all the data available are leveraged to better characterize the complexity of tobacco exposure.

In the s-FCM model, we did not include variables that directly assessed environmental tobacco smoke exposure during pregnancy, rather we relied on the maternal and neonatal biospecimen results to indirectly reflect these environmental effects. In post-hoc analyses, we reran the s-FCM models and added self-reported number of smokers in home during pregnancy and daily partner smoking amount in the presence of the participant to index environmental tobacco smoke exposure incurred by the mother during pregnancy. s-FCM results indicated some influence, albeit small, on group classification. Specifically, there was an increased spread of the non-exposed subjects around the Sammon mapping centroid as

would be expected, but the number of subjects best classified in the non-exposed group remained the same. Between the lighter-exposed and heavier-exposed groups, classification also was highly stable, where only two subjects were re-classified from lighter-to heavier-exposed in the models that also included the two environmental variables. However, the *exposure inconsistency rate* declined by 20%, and the optimization procedures indicated that the environmental variables could be removed from the model without decreasing the *exposure accuracy rates*. Therefore, following s-FCM optimization procedures, the original model without the added two environmental measures was retained as the most parsimonious. Although these findings and other work suggests [5,30] that environmental exposure contributes to tobacco exposure topography, its incremental effects, at least modeled by the two environmental variables included here, beyond cotinine level in maternal urine and neonatal meconium was modest.

Unfortunately, a major obstacle of s-FCM is its innovative nature and related computational requirements. Currently, s-FCM is written in Matlab [2], which is not suitable for all applications and not easily accessible to many non-statistician biomedical researchers. To make s-FCM more available and accessible, we plan to design a user-friendly online software program. In future studies, there might also be enhanced value in integrating the s-FCM with calibration approach [22,23]. Using these methods in conjunction with one another might capitalize on their respective advantages and help further tobacco exposure research. The implication of our method could be far reaching, as this s-FCM technique is highly applicable in characterizing other drugs of abuse over pregnancy as long as important exposure-related variables are measured.

5. Conclusions

The proposed fuzzy clustering approach modeled the exposure-related attributes collected from our sample, and thereby utilized the full information reflected in the repeatedly measured exposure variables, including nicotine constituents of preferred cigarette brand, Fagerstrom nicotine dependence scores, self-reported average cigarettes smoked per day in each prenatal months, and cotinine levels in mothers' urines during pregnancy and neonates' meconium samples. Each variable was useful in providing unique information on tobacco exposure to classify groups of women. By using fuzzy clustering, we empirically integrated multiple sources of data and statistically described patterns of prenatal tobacco exposure. Furthermore, this approach demonstrated incremental utility over traditional approaches by enhancing the characterization of exposure effects on developmental changes of irritability reactivity in neonates in their first month of life. The utility of this statistical approach was strengthened by showing heavier exposed neonates weighed less at birth, consistent decades of research findings [21,24,41,48,66,72].

Acknowledgments

Abbreviations

FCM	Fuzzy c-Means Model
FTND	Fagerstrom Test for Nicotine Dependence
HTE	Heavier-tobacco-exposed
IR	Irritability
LTE	Lighter-tobacco-exposed
MAR	Missing at random
XB	Xie and Beni's index
s-FCM	specially-designed-multiple-imputation-based Fuzzy c-Means Model
TE	Tobacco-Exposed
NE	Non-exposed
MIGM	multiple indicator growth model

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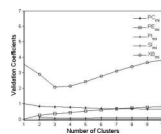**Figure 1.**
Multiple imputation based validation indices PC_m = Partition Coefficient; PE_m = Partition Entropy; PI_m = Partition Index; SI_m = Separation Index; XB_m = Xie and Beni's Index
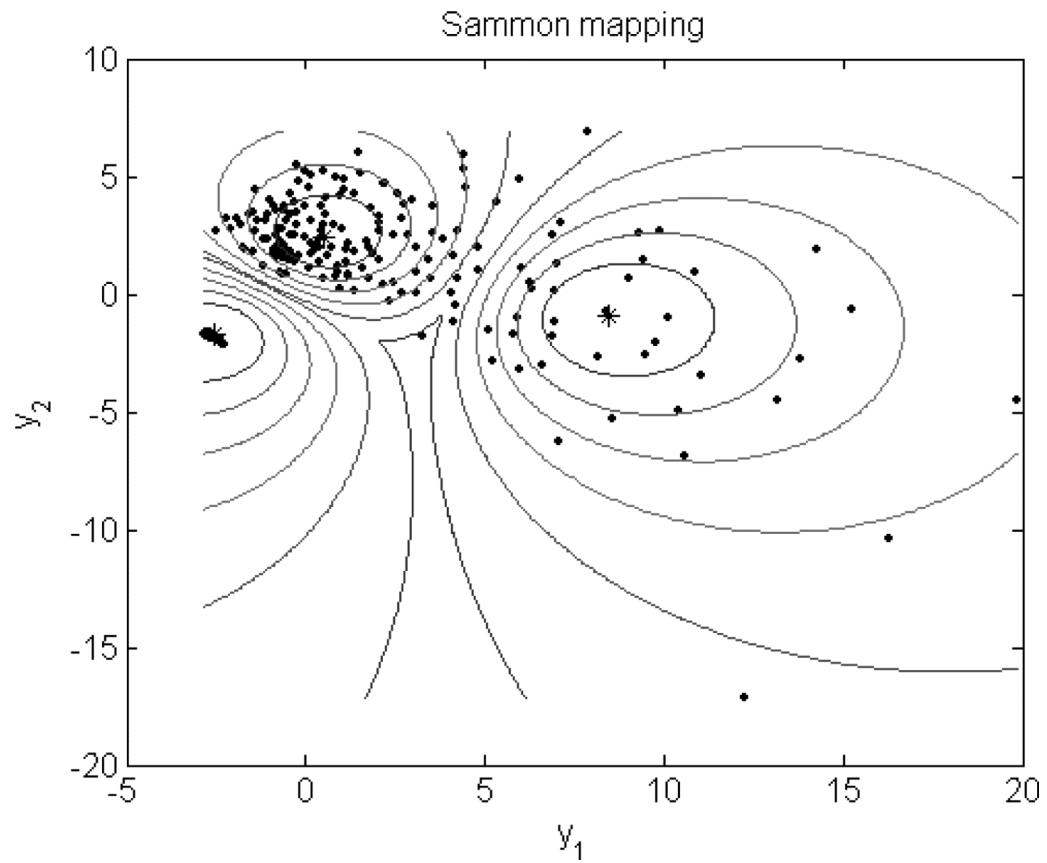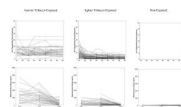
Figure 2.
Sammon mapping of clusters

Figure 3.
Functional curves of self-reported smoking (upper panel, x-axis: M1-9 stands for typical cigarettes/day for months 1-9) and cotinine level in maternal urine samples (lower panel, x-axis: samples taken at 16-, 28- and 40-week interviews) for the tobacco exposure clusters
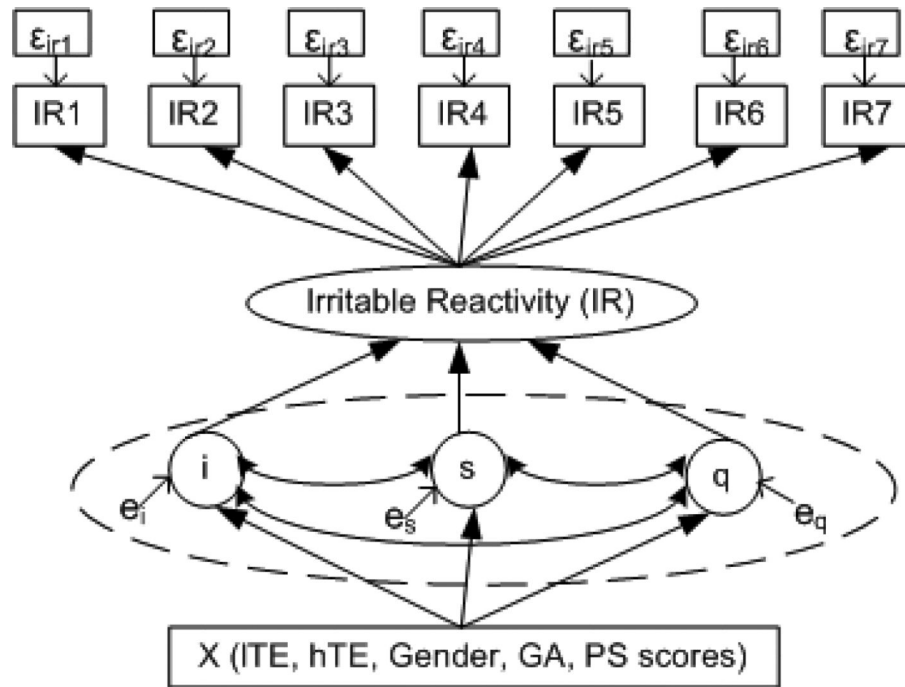
Figure 4.
Multiple indicator growth model for IR using Lighter-Tobacco-Exposed (lTE) and Heavier-Tobacco-Exposed (hTE) groups, where IR1-7 (IR1= Irritability to visual stimuli; IR2 = Irritability to auditory stimuli; IR3= Irritability to handling; IR4= Irritability to reflex elicitation; IR5= Latency to soothe after Moro reflex; IR6= Soothability after reflex elicitation; IR7= Rated reinforcement value) are IR indicators associated with errors ε_{ir1-7} and latent growth parameters (i, s, q) associated with errors $e_{i,s,q}$ are regressed on X

Figure 5.
Interation of hTE and neonate sex on IR

Table 1

Differences in smoking-related variables that were used to charaterize tobacco exposure between lighter Tobacco-Exposed (lTE) and heavier Tobacco-Exposed (hTE) neonates

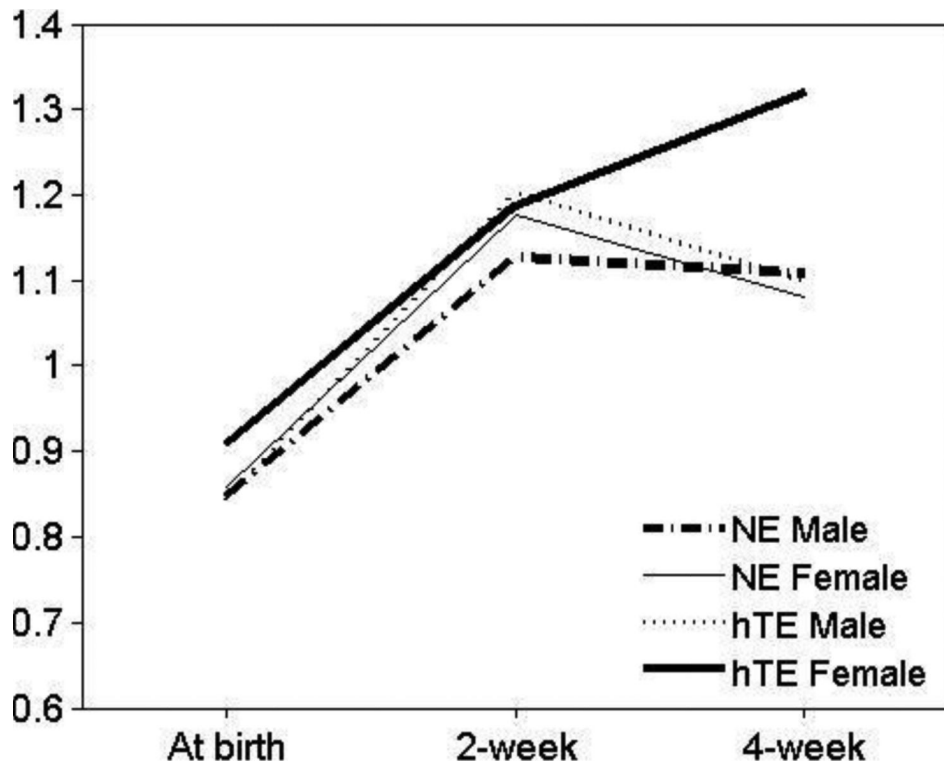|	lTE		hTE	
	M	*SD*	*M*	*SD*
Cotinine level (ng/mL):				
Maternal urine				
16 weeks[***]	120.67	225.18	1165.17	844.89
28 weeks[***]	134.61	25.64	1063.05	686.30
40 weeks[***]	22.01	46.66	279.16	308.68
Neonate meconium				
At delivery[**]	49.44	180.60	787.90	1736.72
Nicotine in Brand (mg)				
16 weeks	0.94	0.27	1.02	0.27
28 weeks[*]	0.94	0.27	1.03	0.24
At delivery[*]	0.94	0.27	1.03	0.25
Self-reported Typical Smoking (cigarettes/day)				
Month 1[***]	4.95	5.24	14.79	6.43
Month 2[***]	2.11	2.92	12.70	6.10
Month 3[***]	1.49	2.25	11.75	5.98
Month 4[***]	1.47	2.47	12.87	6.49
Month 5[***]	1.29	2.28	12.80	6.67
Month 6[***]	1.21	2.00	12.42	7.03
Month 7[***]	0.98	1.83	12.76	6.78
Month 8[***]	0.89	1.68	12.49	6.79
Month 9[***]	0.83	1.57	12.13	6.74
FTND[+]				
Item 1	0.17	0.38	0.13	0.34
Item 2[***]	0.44	0.50	0.93	0.27
Item 3[***]	0.18	0.39	0.50	0.51
Item 4[**]	0.22	0.42	0.43	0.50
Item 5[***]	0.24	0.51	0.98	0.58
Item 6[***]	0.61	1.06	2.03	0.97

Note.

FTND = Fagerstrom Test of Nicotine Dependence [33] Item 1: Do you find it difficult to refrain from smoking in places where it is forbidden?(No = 0; Yes =1) Item 2: Which cigarette would you hate most to give up?(The first in the morning = 1; Any other = 0) Item 3: Do you smoke more frequently during the first hours after awakening than during the rest of the day?(No =0; Yes =1) Item 4: Do you smoke even if you are so ill that you are in bed most of the day?(No=0; Yes =1) Item 5: How many cigarettes per day do you smoke? (10 or less =0; 11-20 = 1; 21-30 =2; 31 or more = 3) Item 6: How soon after you wake up do you smoke your first cigarette? (After 60 minutes = 0; 31-60 minutes =1; 6-30 minutes =2; within 5 minutes =3). Item 6 was recoded to be consistent with all other items scores where higher scores reflect greater nicotine dependence. All item scores were averaged across visits.

*
 p < 0.10

**
 p < 0.05

***
 p < 0.001

Table 2

Descriptive statistics on background variables between lighter-Tobacco-Exposed (lTE) and heavier-Tobacco-Exposed (hTE)

	lTE		hTE	
	M / %	SD	M / %	SD
Maternal age at delivery (years)	25.0	4.9	25.8	4.7
Maternal education (years)	13.1	1.6	12.1	1.2
%Medicaid	83	--	93	--
%Married	36	--	43	--
Maternal Race/Ethnicity				
(%White, non-Hispanic)	75	--	85	--
Maternal Weight Gain (lbs)	36.7	19.1	30.8	21.1
Number of Previous Pregnancies[*]	1.5	1.9	2.5	2.5
Healthy Diet[1]	4.42	0.69	4.22	0.68
Exercise (3 times/week)				
%Pre-pregnancy	50	--	35	--
%16 weeks[*]	34	--	23	--
%28 weeks	45	--	33	--
%Delivery	34	--	20	--
%Prenatal Marijuana Use	20	--	18	--
Average Number of Alcohol Drinks/Day				
Month 1 pregnancy[**]	0.287	0.431	0.080	0.146
Month 2 pregnancy[*]	0.039	0.127	0.002	0.008
Month 3 pregnancy[*]	0.008	0.041	0.001	0.002
Month 4 pregnancy	0.003	0.010	0.001	0.008
Month 5 pregnancy[**]	0.004	0.013	0.000	0.000
Month 6 pregnancy	0.005	0.017	0.001	0.009
Month 7 pregnancy[*]	0.006	0.020	0.001	0.006
Month 8 pregnancy[*]	0.006	0.030	0.000	0.000
Month 9 pregnancy[*]	0.007	0.032	0.000	0.000
Anti-depressants	13	--	8	--
Opioid-based Analgesics	20	--	33	--
Asthma	5	--	5	--
Thyroid	2	--	--	--
Estimated Maternal Intelligence[2]	95.76	11.69	92.51	10.63
BSI Subscale T score[3]				
Anxiety	51.02	9.53	49.53	9.94
Depression	53.77	8.74	53.43	8.82
Hostility	57.59	9.37	57.10	8.41
Interpersonal Sensitivity	53.88	9.15	51.08	8.76

	lTE		hTE	
	M / %	**SD**	**M / %**	**SD**
Obsessive-Compulsive [**]	58.04	10.46	52.78	10.80
Paranoid Ideation	53.00	9.35	51.08	8.19
Phobic Anxiety	51.45	8.71	52.23	7.86
Psychoticism	55.22	9.37	56.05	10.04
Somatization	59.18	8.86	58.08	8.17
CAARS Subscale T score[4]				
Hyperactivity	48.20	8.17	47.18	8.90
Impulsivity	46.29	7.24	45.68	5.62
Inattention	48.44	8.39	46.53	8.34
%Diabetes	5	--	13	--
Delivery				
%Spontaneous vaginal	45		33	
%Induced vaginal	25	--	33	--
%Caesarean & other extraction	30	--	35	--
%Heart Disease	3	--	5	--
%Anemia	11	--	18	--
%Infections	9	--	10	--
%Toxemia/preeclampsia	9	--	18	--

Note.

[*] $p < 0.05$

[**] $p < 0.01$.

[1] Healthy diet represents an average score of each subject across 3 visits if reported consumption of tuna, fish, bread, fruit, vegetables and dairy (1=yes, 0=no).

[2] Woodcock-Johnson III Brief Intellectual Ability [73].

[3] BSI = Brief Symptom Inventory [19]

[4] CAARS = Connor's Adult ADHD Rating Scale – Short Form, Self-report [16].