Is Kangaroo Mother Care Associated with Reducing the Odds of Autism Spectrum Disorder in preterm babies in Canada?
A Protocol for a Longitudinal Quasi-experimental Study

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Scientific Abstract

Background and Research Aim

According to the 2015 data from the National Autism Spectrum Disorder Surveillance System (NASS), 1 in 66 Canadian children is diagnosed with Autism Spectrum Disorder. The risk of Autism spectrum Disorder (ASD) is elevated by approximately 70% in preterm babies born between 28 to 37 weeks of gestational age. Kangaroo mother care, a new technique that involves placing the infant upright on the mother's bare chest to provide skin to skin contact between the mother and the infant, has been reported to have a wide range of beneficial effects including improved brain and motor development, cognitive functioning, immunity, and physiologic stability [1]. Previous studies that examine the association between KMC and cognitive functioning mostly focused on examining the Intellectual Quotient(IQ) as the outcome; however, other studies suggest that almost half (44%) of children identified with ASD has average to above average intellectual ability [2]. Few studies have been conducted to examine the preventive effect of KMC on the outcome of Autism spectrum Disorder in premature babies (gestational age less than 37 weeks) who run an elevated risk in developing ASD in later childhood. The aim of this study is to gain insight into the relationship between Kangaroo Mother Care and the outcome of Autism Spectrum Disorder and further estimate the magnitude of the association.

Methods

A longitudinal quasi-experimental study will be conducted to assess the association between Kangaroo Mother Care (KMC) and Autism Spectrum Disorder (ASD). In this study we will recruit a total of 4238 preterm babies between 28 to 37 weeks gestational age, 2119 in intervention group and 2119 in control group. Subjects in the intervention group will receive Kangaroo Mother Care while those in the non-intervention group will receive traditional care. Each case will be recruited from St. Michael's hospital and Sunny Brook Health Science Center. The two intervention hospitals will be matched by geographical proximity and level of neonatal care to two non-intervention hospitals, Mt. Sinai hospital and North York General Hospital, from which controls would be recruited. Each case will be matched to a control by sex and gestational age. Demographic data including parents' educational and income level as well as ethnicity will be recorded when the child is born in order to control for potential confounders at the analysis stage. The total amount of time KMC being implemented will be recorded for each case by NICU nurses. After subjects are discharged from the NICU in each hospital, both cases and controls will be followed and screened starting their second birthday until they reach ten years old. Subjects will be screened annually during the follow-up period and referred to a comprehensive diagnostic evaluation if their screening results were positive. Diagnosis of ASD will be based on DSM-5. The Modified Checklist for Autism in Toddlers, Revised (MCHAT-R) will be used as the screening tool before subjects the age of three; afterwards, the childhood Autism Spectrum Test (CAST) will be adopted. Finally, logistic regression will be used to assess the intervention of Kangaroo mother Care based on the total amount of time (length of time per day added up over the entire duration of the KMC) the KMC was implemented.

Expected Outcomes
We hypothesize that that Kangaroo Mother Care can reduce the odds of Autism Spectrum Disorder (ASD) in preterm babies in the Canadian population compared to traditional care after adjusting to all possible confounders.

Lay Summary

Autism Spectrum Disorder (ASD) can have devastating effects throughout life. People with ASD suffer impairment in social communication skills and language development. According to the 2015 data from the National Autism Spectrum Disorder Surveillance System (NASS), 1 in 66 Canadian children is diagnosed with Autism Spectrum Disorder. The risk of ASD is elevated by approximately 70% in preterm babies born between 28 to 37 weeks of gestational age. Kangaroo mother care, a new technique that involves placing the infant upright on the mother's bare chest to provide skin to skin contact between the mother and the infant, has been reported to have beneficial effects on cognitive functioning. In this study, we are studying the relationship between the intervention of KMC and the outcome of ASD in preterm babies. To understand the relationship, we will recruit 4238 preterm babies between 28 to 37 weeks gestational age, 2119 in intervention group and 2119 in control group. These subjects would be recruited either in the intervention or non-intervention group based on where they were given birth. The total amount of time spent on KMC will be recorded as the intervention variable for those who are in the intervention group, while those in the non-intervention group will receive traditional care, in which babies are placed in incubators. After subjects are discharged from the NICU in each hospital, they will be followed up starting their second birthday until they reach ten years old. Subjects will be screened annually in the follow-up period and referred to a comprehensive diagnostic evaluation if screening results were positive. Two different screening tools will be adopted considering the age of the subject being screening. By analyzing the data collected on the exposure and outcome variable from recruited subjects, we hope to identify the preventive effect provided by KMC, the skin to skin contact between mother and her preterm baby, on the outcome of ASD, and raise awareness of the importance of Kangaroo Mother Care.

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Statement of Problem

Kangaroo mother Care is a perinatal intervention involving skin-to-skin contact between the preterm or low birth weight infants [3]. It was first implemented in low developed countries where mortality of premature babies was very high because of the unavailability of incubators, hospital facilities and well-trained health care providers. For the preterm infants in these areas, Kangaroo Mother Care becomes an effective way to provide the warmth, protection, intimacy, safety, and stimulation the baby needs in early developmental stage [3]. Since its introduction in the late 90s, various studies have already shown that Kangaroo Mother Care has had beneficial results for babies, including positive effects on neurocognitive development.[4] According to mammalian neuroscience, the intimate mother-infant contact that simulates the environment in the mother's uterus before labour evokes advances in neuron connections, ensuring fulfillment of intrinsic biological needs [5]. The time right after birth is said to represent a "psycho-physiologically sensitive period for programming physiology and behaviors in the future" [5]. Also, the third trimester which preterm babies lack is a key development period for the central nervous system of babies[4]; therefore, the KMC is most likely to have a significant and durable effect on cognitive functioning in these babies [4]. Despite its wide range of advantages, KMC is not widely implemented in developed countries including Canada due to lack of clinical guidelines, structured protocols, and trained staff. In traditional care, newborns are separated from their mother as the baby constantly stay in an incubator for protection; thus skin-to-skin contact is very limited. There is currently still no official estimate of how prevalent Kangaroo Mother Care is in Canada; however, recently, more and more health care providers have started to emphasize the importance of skin-to-skin intimacy between the baby and the mother, as 34 hospitals in Canada including St. Michael's Hospital and Sunny Brook Health Science Center have joined the worldwide "Kangaroo Challenge" to raise the awareness of KMC.

In Canada, 7.8% of neonates are born premature [6]. While differences in premature birth rates vary between provinces, Ontario has a preterm birth rate of 8.1% [6]. Premature infants (born less than 37 weeks gestational age) run a higher risks of developing Autism Spectrum Disorder and are often at risk of developmental delays [7]. Clinical research have shown that the prevalence of autism increases with every week short of 37 weeks of gestation. The autism prevalence among children born with typical gestation (37 to 41 weeks) is 1.2%. This prevalence rises to 2% for those born at 27 to 33 weeks of gestation and to nearly 4% for those born at less than 27 weeks of gestation [8].

Autism Spectrum Disorder is a neurological and developmental disorder characterized by a wide range of conditions, such as difficulties with social and communication skills as well as restricted and repetitive behaviors [9]. It is a disease outcome that the exact cause is not known; however, genetic or environmental risk factors might both have an effect [10]. The number of children diagnosed with ASD has been rapidly rising in the past few years, with 1 in 66 Canadian children is diagnosed with Autism Spectrum disorder based on the 2015 data from the National Autism Spectrum Disorder Surveillance System (NASS). According to an ASD study in children aged 8 years in United States in 2014, the median age of earliest known ASD diagnosis for all subtypes combined was 52 months, ranging from 40 months to 59 months [2]. Also, other research has shown that a diagnosis of ASD at age 2 can be reliable, valid, and stable [11].

The potential benefits of Kangaroo Mother Care (KMC) in babies is presented widely in research studies, including improved brain and motor development, cognitive functioning, immunity, and physiologic stability [1]. Previous studies that examine the association between KMC and cognitive functioning mostly focused on examining the Intellectual Quotient (IQ) as the outcome; however, other studies suggest that almost half (44%) of children identified with ASD

has average to above average intellectual ability [2]. Few studies have been conducted to examine the preventive effect of KMC on the outcome of ASD in premature babies (gestational age less than 37 weeks) who run an elevated risk in developing ASD in later childhood.

The aim of this study is to gain insight into the relationship between Kangaroo Mother Care and the outcome of Autism Spectrum Disorder and further estimate the magnitude of the association. We hypothesize that that Kangaroo Mother Care can reduce the odds of Autism Spectrum Disorder (ASD) in preterm babies in the Canadian population compared to traditional care.

Methods

1. Study design

A longitudinal quasi-experimental study with intervention without randomization will be conducted in this study. Since the eligibility of well-trained staff for Kangaroo Mother Care are not the same across hospitals, it would be unfeasible for to undergo a true randomized experiment within one hospital. The study does not involve manipulating the intervention, while the intervention will be determined by which hospital the mother end up giving birth to the baby. Therefore, a longitudinal quasi-experimental study will be used to examine the association between the intervention of Kangaroo Mother Care and the outcome of Autism Spectrum Disorder. The study design enables us to examine how the length and duration of Kangaroo Mother Care might have influenced the result of the outcome of Autism Spectrum Disorder and it also allows us to follow our study subjects for a complete period of time since their birth until the age of 10 when ASD cases should likely have already manifested. By following participants through a 10-year period longitudinal quasi-experimental study, we will be able to calculate the odds ratio of the Autism Spectrum Disorder in the intervention group compared to the non-intervention group.

2. Study setting and participants

This study is a longitudinal quasi-experiment conducted in two sets of hospitals in the Greater Toronto Area, which is four hospitals in total. One set is located at North York while the other located at downtown Toronto. The intervention group will be recruited from St. Michael's hospital and Sunny Brook Health Science Center; the non-intervention group will be recruited from the Mount Sinai Hospital, and North York General Hospital. The hospitals in which cases and controls will be recruited are matched on level of neonatal care, with all of them having appropriate level one to four NICU (Neonatal Intensive Care Unit) facilities. Approximately 6000 newborn babies are born annually in each of the four hospitals. Geographical proximity is also matched in order to control for a range of confounders including income level, mother educational level, and ethnicity.

St. Michael's hospital and Sunny Brook Health Science Center are two of the hospitals which participated in the "Kangaroo Challenge 2019". Since all preterm babies are required to stay in the Neonatal Intensive Care Unit (NICU) for protection, the NICU staff members there are trained and encouraged to adopt Kangaroo Mother Care as an integral therapy for premature infants with institutional protocols on KMC. (The "Kangaroo Challenge" is a worldwide competition aiming to raise awareness and improve the implementation of kangaroo mother care.) While Mt. Sinai hospital and North York General Hospital are not participants in the "Kangaroo Challenge", traditional care is given for preterm babies in their NICU. Participant recruitment and the assessment of the initiation, length and duration of Kangaroo Mother Care will be conducted in St. Michael's hospital and Sunny Brook Health Science Center. Follow-up appointments for both cases and controls will be scheduled around their preference date and

location, with their parent/ guardian, and our researcher present.

Inclusion criteria
Infants will be included if they met the following criteria: (1) born more than 28 weeks but less than 37 weeks gestational age (2) vital signs of the infant is stable. The definition for stable vital signs for neonates is determined as follows: (a) Auxiliary temperature of 36 to 38 degrees Celsius. (b) Heart rate of 93 to 182 per minute (c) Respiratory rate 40 to 60 per minute (d) Mean arterial blood pressure (mmHg) > postmenstrual age (e) Oxygen saturation rate(SpO2) > 90%

Exclusion criteria
Infants will be excluded from the study if they had any of the following situation: (1) present with disease and would be too ill to leave the incubator (2) under breathing support such as a mechanical ventilator (3) undergoing a major surgery/ procedure in the next 24 hours (4) mother of the neonate is not under stable health condition or is ill with infectious disease

3. Variables
Intervention- Kangaroo Mother Care (KMC)
The Kangaroo Mother Care (KMC), defined according to WHO guide for Kangaroo Mother Care (KMC), is a perinatal intervention defined as “frequent skin-to-skin contact between mother and her newborn baby” [3]. It is a new technique that involves placing the infant upright on the mother’s bare chest. The mother should wear a gown that is open on the chest while the infant should only wear a diaper with a blanket covered to help in maintaining thermal homeostasis. According to the WHO guide, Kangaroo Mother Care should be gradually initiated as the length of time increases until at least one hour a per practice to as continuous as possible.[3] The duration usually lasts until the baby reaches full term or until the infant shows behavioral manifestations that they are ready for separation.[3] In our study, the practice will be recorded as ended once the infants are discharged from the hospital. Also, KMC should be conducted under a room temperature around 25 degree Celsius within a private and quiet space.[3] Breastfeeding is not considered a required component of KMC in our study. The ascertainment of KMC in the intervention group would be recorded by hospitals nurses and documented in a database created by our research team. The total amount of intervention would be calculated by adding up the length of time each day over the entire duration of the KMC implemented.

Outcome-Autism Spectrum Disorder (ASD)
The diagnosis of Spectrum Disorder will be based on the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5). According to DSM-5, Autism Spectrum Disorder is characterized by “severe deficits in either verbal or nonverbal social communication skills that cause severe dysfunctions”, or “specific repetitive patterns of behavior, interests, or activities”, demonstrated by at least two of the following: “Stereotyped or repetitive motor movements”; “Insistence on sameness inflexible adherence to routines, or ritualized patterns or verbal nonverbal behavior”; “Hyper- or hypo-reactivity to sensory input or unusual interests in sensory aspects of the environment” [9]. These symptoms have to appear in early development period and should lead to clinically significant impairment which could not be better explained by intellectual disability in social or other important fields [9].

To obtain information on the study subjects and pre-screen for the possibility of ASD in a child, the parent or guardian of the study subject will be requested to answer questions in the following questionnaires in a scheduled follow-up interview at the original hospital where the child was recruited with our research staff. They will answer the questions on the questionnaire addressed by the interviewer while the information and answer provided will be documented in

an electronic tablet by the interviewer. The interview process should be done by a parent or guardian that is in a close relationship with the child and understands the child well.

The Modified Checklist for Autism in Toddlers, Revised (M-CHAT-R) is a widely used parental screening questionnaire that consists of 23 yes/no questions that assess the sensory responsiveness, early language and communication, social relatedness, and early joint attention in a child [12]. The M-CHAT-R would be used when the child is aged between 16-36 months. According to the checklist, a score between 0-2 indicates low risk, yet a follow-up re-screening is required only after a year. A score between 3-7 suggests medium risk while administering a subsequent second stage of follow-up test using the Modified Checklist for Autism in Toddlers, Revised with follow-up (M-CHAT-R/F) to get additional information about at-risk responses is required [12]. If M-CHAT-R/F score remains at 2 or higher, the child has screened positive. Scores between 8-20 in M-CHAT-R shows high risk and the child would be screened negative only if the score on M-CHAT-R/F is between 0-1 [12].

The childhood Autism Spectrum Test (CAST) developed by the Autism Research Centre, University of Cambridge is valid for children aged from 3 to 11 will be used starting the third year until the end of the study. Scores between 0-14 means low risk for ASD while re-screening using the same questionnaire is required in the following year. Scores above 15 indicates that it is very likely that the child has developed Autism Spectrum Disorder and should be referred to a physician for diagnostic evaluation [13]. All children who are screened positive through questionnaires would further be referred to a clinic with well-trained physician in collaboration with our research team for clinical diagnosis based in DSM-5 criteria. Both referrals to physicians for clinical diagnosis as well as certified diagnosis will be documented.

The M-CHAT-R has shown fairly good reliability and validity in evaluating child autism symptoms in recent studies. A study in the validity of M-CHAT-R showed a sensitivity of 52%, a specificity of 84%, a positive predictive value (PPV) of 20%, and the negative predictive value (NPV) of 96%. False positive and false negative rates were high among children who had hearing and vision impairments. High false positive rates were also associated with lower SES, motor and cognitive impairments, and emotional/behavioral dysregulation at age 2 [14]. The CAST has also shown good test–retest reliability and has proven to be a reliable and valid test for screening for ASD in a Spanish research [15]. In this paper, the questionnaire showed higher values of sensitivity (83.9%) and specificity (92.5%) at the cut-off score of 15 ($PPV = 0.63$). These results are very close to those obtained by another UK research [16].

Potential confounders and bias

Measurement bias involving either over or under-diagnosis is possible considering that diagnostic data is not always accurate because it is subject to human influence. Over-diagnosis or under-diagnosis of Autism Spectrum Disorder (ASD) can be a big issue since the symptoms and patterns vary widely among children and diagnosis will be based on physician and parent observation of the child. We will try to minimize this measurement bias by ensuring rigorous and regular follow-up interviews among participants in the cohort. Information bias will be addressed by making sure the questions asked in the follow-up interview session is well-articulated and explained by our interviewers while all parents whose child is participating in the study fully understand the definition and possible symptoms and behaviors in children with ASD. Other potential confounders including parents' educational and income level as well as ethnicity would be recorded when the child is born [17]. However, matching cases and controls by sex and

gestational age as well as the area of hospital in which they were born enables us to minimize a range of potential confounding mentioned above.

Bias that might occur during analysis stage involves suspecting outcome based on intervention, which is referred to as the diagnostic suspicion bias. We will control for this by blinding interviewers responsible for collecting questionnaire data and physicians responsible for diagnosis. The research data analyst will also be blinded.

4. Recruitment and follow-ups

Infants admitted between September 2019 and September 2021 in either St. Michael's Hospital, Sunnybrook Health Science Center who meet the inclusion criteria but not the exclusion criteria would be recruited as cases. Each case recruited at St. Michael's hospital will be matched to a control considering gender and gestational age of the baby at Mount Sinai hospital, and every case identified at Sunny Brook Health Science Center would also be matched by gender and gestational age to a control at North York General Hospital.

Those recruited at St. Michael's Hospital or Sunnybrook Health Science Center will be the intervention group as they receive KMC, while those chosen at Mount Sinai Hospital or North York General Hospital will serve as the control group as they receive traditional care. After the baby reaches full term, he will be discharged from the hospital as both the kangaroo mother care and traditional care ends when they leave the hospital.

Two years after, the parent or guardian of all of the babies that survived after two years after birth from the initial cohort will be contacted for a follow-up interview scheduled around their preference dates and location. The interview will be guided by our researcher as the parent/ guardian will answer a series of questions about their child. The assessment will take around one hour as our researchers explain the objective the questionnaire first and subsequently start the interview. The Modified Checklist for Autism in Toddlers, Revised (M-CHAT-R), the Modified Checklist for Autism in Toddlers, Revised with Follow-up (M-CHAT-R/F), and the childhood Autism Spectrum Test (CAST) are used for screening. The details of the questionnaires are presented in the outcome measures section below. Follow-up evaluation on the child would be conducted at annual intervals by interviewing their parent/ guardian after the child's 2 year-old birthday until the age of 10 before they reach adolescence in September 2029 or 2031. Parents are also encouraged to seek our research team for help on their own initiative once they recognize abnormality in their child and would like to have their child screened. Various measures will be addressed to minimize loss of follow-up, including the following: (1) Obtain contact information for each subject as well as alternate contacts; (2) Discuss with patients the importance of returning for all follow-up visits; (3) Schedule follow-up appointments around patient preferences; (4) Continue trying to contact lost patients and all alternate contacts; (6) Have staff at research center to help locate subjects. Subjects moving out of Toronto will be a reason for us to exclude participants.

5. Procedure and steps of the Kangaroo Mother Care intervention

Kangaroo Mother Care should be administered in the NICU or in a special care neonatal ward with a trained nurse present [18]. The mother should be fully informed of how Kangaroo mother care will be implemented and be physically and mentally ready. The initiation of skin-to-skin contact should start gradually with a smooth transition from a traditional care in a incubator to Kangaroo Mother Care guided by a fully-trained nurse since it takes time for babies to adapt to a new type of care [3]. Every time when the Kangaroo Mother Care is initiated, it should last for at least one hour with room temperature controlled at 25 degrees Celsius [3]. The length of

every practice should always be documented by the mother and collected by the nurse from the commence date to the end date. The steps are as follows:[18]

(1) Prepare the mother for Kangaroo Mother Care:
Mother should wear a gown open on the chest. Mother should be aware that the care will take at least one-hour. Mobile phones and any related technology products will not be allowed.
(2) Check the environment:
Provide adequate privacy (by screens or curtains). Ensure a calming environment with a low level of sound with room temperature of 25 degrees Celsius.
(3) Check if the infant is under stable condition for intervention according to conditions listed in inclusion and exclusion criteria. The infant should only wear a diaper with a blanket covered on his back.
(4) Adjust to the Kangaroo position: the mother can either sit on a chair or lie down in a bed. Breastfeeding during KMC is not required but encouraged.
(5) Supervise over babies:
During KMC, vital signs should be continuously monitored by the mother and reported to trained nurses once they recognize signs of instability on their baby. KMC should be ended in time when the mother requests to or when the condition of the baby or mother does not allow it to continue.
(6) Record length after practice every time:
For the purpose of studying the association between the intervention of Kangaroo mother care and the outcome of Autism Spectrum Disorder, the length of every practice every day must be recorded with the date by a nurse in an electronic database created by our researchers. If it was the last practice before the infant is discharged from the hospital, it should also be labeled.

6. Data collection and management

Data collection is performed by our researcher after each case is discharged from the hospital. A password protected electronic online database will be created for St. Michael's Hospital and Sunny Brook Health Science Center for data collection on the information of length, duration, and date kangaroo mother care practice for every case. General information including the name of the child's mother, sex of the child, residence address, email, and cell-phone number. The ID number and name of the child with also be collected afterwards. Only responsible NICU nurses and our researcher staff that have signed confidentiality agreement will be able to access the database while only the nurses will have authority for data entry. Each responsible nurse and researcher and will be given a computer-generated id number as an account so that our research team will be able to track all the actions made in the database. The complete data of each case will be updated after the baby is discharged from the hospital.

Also, another password protected online database with standard format questionnaires including M-CHAT-R, M-CHAT-R/F, and CAST for each case and control will be established for the purpose of collecting data on Autism Spectrum Disorder screening results. Only researchers responsible for interview will be given a computer-generated account number as an access for data entry. Our research data analysts will also be given access to extract data in the analysis stage. After all the follow-up is done, all personal identifying information in all databases will be stored separately on a secure server with all the data encrypted.

7. Data analysis

The data collected would be analyzed using logistic regression to examine the odds ratio in terms of the intervention of Kangaroo Mother Care and the outcome of Autism Spectrum

Disorder between the intervention group and non-intervention group. Logistic regression will be used to assess the intervention of Kangaroo mother Care based on the total amount of time (length of time per day added up over the entire duration of the KMC) the kangaroo mother care was implemented. A statistical significance level of 5% will be adopted in all statistical test as p-values <0.05 will be considered significant. Bivariate analyses will be conducted to compare the distribution of potential confounders between the intervention and non-intervention group. The Pearson's chi-squared test would be used to assess goodness of fit.

Other predictors including age of the mother, ethnicity of the baby, mother educational level, income level would be added to the logistical model one at a time. The effects of these covariates will be tested for their significance with the Analysis of Variance (ANOVA) and effect modification. Since fitting too many predictors in one model may result in overfitting, a validation test using standard bootstrap will be conducted to find whether the amount of overfitting should be concerned. Collinearity between the independent variables in our fitted model will be assessed, considering variance inflation factor (VIF) values larger than 10 evident. Collinearity should be dealt with if any variance inflation factors were larger than 10 since collinearity could cause false estimated beta coefficients.

Missing data would be dealt with different valid analyses based on the characteristic and mechanism of it. Complete case analysis, and single and multiple imputation would be used to address data missing completely at random. Multiple imputation would be used analyze data missing at random, while for data missing not at random, a sensitivity analysis would be conducted.

Influential observations will be analyzed to see if extremely outlying data points were present and possibility of data entry error would simultaneously be investigated in the original database. However, if the outlier was not due to data entry error, it will be still be included in the analysis.

Sample size

Estimates of incidence of ASD for the intervention and non-intervention group were entered into a sample size calculator on the ClinCalc website. The formula was adopted from the textbook: 7th edition of Fundamental of Biostatistics. [19] An incidence of 1.2% for the intervention group and 2.4% for the non-intervention group was used. An alpha value of 0.05 was adopted along with 80% power. Results show that a total of 3852 subjects is required with 1926 in each arm. Since expected loss of follow-up is 10%, sample size was increased to 4238. Therefore, the final sample size of 4238 in total, 2119 in intervention group and 2119 in control group, would be deemed enough to detect a significant effect.

Ethics

Although our study subjects of preterm babies is considered a vulnerable population, the Kangaroo Mother Care intervention poses little to no risk to participating babies and their families since trained nurses with rigorous procedure is demanded as mentioned in our protocol. Parent or guardian of the babies should provide signed informed parental consent prior to engaging in the study. In the informed parental consent form, the following will be addressed: our study purpose; research intervention; procedure they will undergo; duration of the study; risks and discomforts that might occur; benefits and reimbursements as a result of their

voluntary participation; the contact information of our research staff; how our research team will utilize, share findings, but at the same time maintain the confidentiality of their data and information about their child. Their right to refuse or withdraw from the study will also be included considering the voluntary nature of consent to the study.
Parent informed consent will be signed in the hospitals in which their babies were born once they agree to participate in the study after labour. Parent consents will be documented and sent with our research protocol to their respective hospital for REB review.

Discussion

This study aims at evaluating the effects of Kangaroo Mother Care intervention (KMC) on the outcome of Autism Spectrum Disorder in preterm babies born between the gestational age of 28 to 37 weeks. To this aim, 3852 participants (1926 cases; 1926 controls) will be followed from two years old to ten years old. The results are expected to provide evidence for the effectiveness and the association of the total amount of time KMC was implemented and the odds of Autism Spectrum Disorder in preterm babies, and raise awareness of the importance and benefits of parent-baby skin-to-skin right after birth. Health care professionals should be aware that hospital routines, such as weighing the baby, should not take precedence to the baby's skin to skin contact with the mother.

Unavailability of experienced staff and parent anxiety when their baby leaves the incubator are the common barriers in the implementation of KMC. Also, it is often impossible for many preterm babies, especially extreme preterm ones who require life-support facilities to leave the incubator. Due to this, Kangaroo Mother Care could not be actually implemented to the highest ASD risk group, not to mention being able to see its preventive effect.

Measurement bias would be dealt with by ensuring rigorous and regular follow-up interviews among participants in the cohort. Information bias will be addressed by making sure the questions asked in the follow-up interview session is well-articulated and explained by our interviewers while all parents whose child is participating in the study fully understand the definition and possible symptoms and behaviors in children with ASD. Other potential demographic confounders will be addressed in the adjusted logistic model while taken into account in the analysis stage. Interactions, collinearity, influential observations, missing data, and model validation will also be addressed. However, difficulty in accounting for certain confounders such as mother's diet before labour and family history of ASD could affect the result. Another limitation in this study is that since preterm babies only account for 8.1% of all babies born annually in Toronto, it would be take a lot of time to recruit enough subjects from the four hospitals to reach the sample size required to detect a clinically significant effect. Otherwise, more hospitals sites for recruitment of subjects should be identified to make the recruit process shorter.

Last, loss of follow-up would likely be a big issue due to participants moving out of the Greater Toronto Area. Various methods would be used to maintain contact over the ten year follow-up period, including scheduling follow-up appointments around subject preferences, contact subjects or alternate contacts by phone for follow-up, and having responsible research staff to help locate subjects over the entire follow-up period. Before the study starts, our researcher will also discuss with the patients the importance of returning to follow-up visits.

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