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Letter to Editor

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COVID-19 and Renal Complications in Neonates and Pediatrics

Reza Bahrami[1], Hossein Neamatzadeh[2,3*], Elahe Akbarian[4]

[1] Neonatal Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
[2] Department of Medical Genetics, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[3] Mother and Newborn Health Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[4] Children Growth Disorder Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

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Corresponding author:

Hossein Neamatzadeh

Email:
neamatzadehh@gmail.com

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Dear editor,

Several studies stated that course of COVID-19 in children is considerably better than adults.[1–3] COVID-19 mortality rates in infected children is less than 1%.[4,5] Some studies indicated the importance of renal function surveillance among infected children with COVID-19.[4] However, there was no sufficient data on renal complications in infected children. The available data showed that ventilation in the infected children might be less aggressive and had less systemic involvement and renal dysfunction than adult patients.[5] Normal renal function defined by serum creatinine (SCr) greater than 110 μmol/L or serum urea greater than 7 mmol/L. Shah et al., reported that the infection might be associated with new-onset nephrotic syndrome in the children.[6] A study from northern China evaluated the epidemiological history, clinical manifestations, treatment and the short-term prognosis of 31 infected children (6 months -17 years) from six provinces. The study showed that the clinical manifestations and laboratory examination results are nonspecific in the infected children. Moreover, renal function and blood glucose were normal in the infected children.[7] Other study among nine Chinese infected infants (age ranges: 1-11 months) showed that those infants did not require intensive care and had no serious complications.[8] In a study, Qui et al. described the clinical and epidemiological characteristics of 36 Chinese hospitalized children (age 0–16 years). Their results showed that none of the children had renal dysfunction.[9] Stewart et al., described data of 52 infected children (age 0–16 years) who referred to Great Ormond Street

Hospital for Children NHS Foundation Trust (London, UK). Their data showed that 24 (46%) of those children had a SCr greater than the upper limit of reference interval (ULRI), and 15 (29%) met the British Association of Pediatric Nephrology (BAPN) diagnostic criteria for acute kidney injury (AKI). Moreover, most cases of AKI occurred in those children who admitted to the pediatrics ICU (PICU) and those children with pediatrics inflammatory multisystem syndrome temporarily associated with the infection.[4] Deep et al., have reported that the incidence of AKI in infected children might be between 2% and 3%.[5] But, their reports was less than the epidemiology of 26% renal dysfunction in children admitted to PICU.[10] Moreover, González-Dambrauskas et al., in a multicenter epidemiological study of critically ill children indicated that AKI occurred in 18% of patients. They proposed that pediatric patients with a comorbidity such as congenital heart diseases and congenital renal diseases and renal transplant patients are at higher risk of renal complications.[11]

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Review Article

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Current State of Knowledge about Transplacental Transmission of SARS-CoV-2 Infection

Atiyeh Javaheri[1], Mahmood Noorishadkam[2], Mahta Mazaheri[2,3,4], Ali Dadbinpour[3], Seyed Alireza Dastgheib[5], Reza Bahrami[6*], Mojgan Karimi-Zarchi[7,8], Fatemeh Asadian[9], Hossein Neamatzadeh[2,3]

[1] Department of Obstetrics and Gynecology, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[2] Mother and Newborn Health Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[3] Department of Medical Genetics, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[4] Stem Cell Biology Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[5] Department of Medical Genetics, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
[6] Neonatal Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
[7] Endometriosis Research Center, Iran University of Medical Sciences, Tehran, Iran
[8] Department of Obstetrics and Gynecology, Iran University of Medical Sciences, Tehran, Iran
[9] Department of Medical Laboratory Sciences, School of Paramedical Science, Shiraz University of Medical Sciences, Shiraz, Iran

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Corresponding author:
Reza Bahrami

Email:
r.bahrami.neo@gmail.com

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ABSTRACT

Background: To date, some cases of perinatal transmission of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) have been reported. However, it is unanswered if these occurred via the trans-placental or the trans-cervical route or through environmental exposure.

Methods: To address this question, we conducted this study to review the current state of knowledge about the transplacental transmission of COVID-19.

Results: There are no known placental findings associated with the COVID-19 infection. The possibility of intrauterine infection has been based mainly on the detection of IgM in the neonatal blood. Real time-PCR tests on amniotic fluid, placenta, and cord blood are required to ascertain the possibility of intrauterine vertical transmission.

Conclusion: There is currently no sufficient and convincing evidence about the transplacental transmission of SARS-COV-2 infection in pregnant mothers. However, the paucity of placental expression of ACE-2 involved in the cytoplasmic entry of SARS-CoV-2 may explain its relative insensitivity to transplacental infection.

Introduction

SARS-CoV-2 infection, the first pandemic of the century, causes the new coronavirus disease (COVID-19). Since the first case of a COVID-19 infection was detected in Wuhan (Hubei Province, China), China, a series of confirmed cases of the COVID-19 were found globally.[1-5] After the SARS-CoV epidemic which in 2003 caused outbreaks in six countries, some studies revealed that the infection led to some adverse outcomes in pregnant women, such as spontaneous pregnancy loss, preterm delivery, and restricted intrauterine growth (IUGR).[6] However, a matched study comparing the clinical course and outcomes of pregnant mothers infected with SARS-CoV with non-pregnant mothers found that pregnant and nonpregnant women had similar clinical symptoms and presentation, but that pregnant mothers had evidence of more severe symptoms of SARS.[7] Moreover, the association between adverse pregnancy outcomes and physiopathological changes connected to the Middle East respiratory syndrome (MERS) was reported.[8] Recent data showed that COVID-19 may be associated with a higher rate of caesareans (CS), preterm births, miscarriage, stillbirth and the virus may be able to cross the placenta to a fetus.[9-11] However, it is unclear yet whether these obstetrical outcomes were as a result of the coronavirus.[12,13] Some studies reported that the COVID-19 infection in pregnant mothers may be associated with mild or moderate disease in most cases, with a low morbidity and mortality rate.[14-16]

Evidence showed that the neonatal infection with COVID-19 is usually asymptomatic and detection rates of Real time-PCR (RT) and the interpretation of IgM, IgG and IL-6 antibodies levels in cord and samples of products of conception (placenta, amniotic fluid and umbilical cord blood) are discussed concerning the immaturity of the fetal and neonatal immune system.[17] COVID-19 could be recovered by RT-PCR from nasal and throat swabs, sputum and feces of symptomatic neonates but not from vaginal swabs, amniotic fluid, placenta, cord blood, neonatal blood or mother's milk. A study including 247 deliveries from infected mothers reported that 63 cases were preterm. One in 20 of neonates born to the tested positive for Covid-19, and five of the neonates died. Three of the deaths appear to have been unrelated to the Covid-19, but two of them might have been linked to virus. Another study including 108 infected mothers revealed that 91% of the neonates were delivered by CS. The first case series have described the clinical features and outcomes of pregnancy in infected mothers with COVID-19 in Wuhan, China reported adverse perinatal outcomes including increased risks of miscarriage, preeclampsia, preterm birth, and stillbirth in the infected mothers.[18,19]

There is an imperative need for conclusive data and research on the possibility of trans-placental migration of COVID-19 as well as pregnancy comorbidity is needed.[20] Previously, in a study, we reported that COVID-19 did not transfer vertically from pregnant women to their neonates.[13] Similarly, Silva et al., in a review concluded that there was no convincing evidence for vertical transmission of COVID-19 in pregnant mothers infected during the third trimester of pregnancy, as also reported for COVID-19 infection.[21] However, some studies which examined the placenta for the presence of COVID-19 using molecular, immunohistochemical techniques and electron microscopy have revealed COVID-19 invasion of the placenta, highlighting the potential for severe morbidity among pregnant women with Covid-19.[22] Thus, questions concerning vertical transmission of COVID-19 from infected mothers to neonates remain unanswered. To address this issue, we performed this review to confirm maternal-fetal infection and known mechanisms that COVID-19 virus was used to trans-placental pathogen migration.

Placenta Findings

Yang et al., in a review of 20 studies including 222 neonates using data on umbilical cord blood, placenta, and/or amniotic fluid have summarized the evidence on vertical transmission of COVID-19. They showed that there is no proof to support the intrauterine vertical transmission of COVID-19.[23] In another review based on five studies from Chinese women who were diagnosed with COVID-19 late in pregnancy (3rd trimester), Cheruiyot et al., demonstrated that there is no definitive evidence of intra-uterine vertical transmission of COVID-19 in pregnant women diagnosed in the third trimester.[24] Patanè et al., in a study including 22 infected mothers by COVID-19 who delivered at Papa Giovanni XXIII Hospital, Bergamo, Italy, examined the possibility of vertical transmission of COVID-19. They have described the first report of cases of positive PCR for SARS-CoV-2 in mother, neonate and placental tissue. Two of those neonates, born from infected mothers, resulted positive for PCR of nasopharyngeal swab. The placentas of these two women who delivered infected neonates with positive nasopharyngeal swab showed chronic intervillositis, with presence of macrophages, both in the intervillous and the villous space. Moreover, the immunohistochemical tests demonstrated chronic intervillositis with macrophages CD68+ infiltration. They revealed that the Single-molecule RNA in situ hybridization raises the possibility of direct visualization of the COVID-19 virus, evaluating the molecular target COVID-19 spike protein mRNA while retaining tissue morphology, a feature that is lost in other methods such as PCR. The presence of COVID-19 virus RNA in the syncytiotrophoblast signifies presence of the virus on the fetal side.[25]In a first report, Algarroba et al., examined potential COVID-19 transmission in the placenta using electron microscopy. Their results demonstrated that the COVID-19 virus invasion in placental tissue and placental infection was associated with COVID-19.[26] Mahyuddin et al., reviewed 40 studies of COVID-19 pregnancies to confirm maternal-fetal infection and known protective mechanisms of the placental barrier that prevent transplacental pathogen migration. In the reviewed studies there was no consensus on diagnostic strategy for congenital infection during covid-19 pandemic. In those studies, the molecular RT-PCR assay of neonatal swabs and a wide range of clinical samples including vaginal secretions, amniotic fluid, breast milk and umbilical cord blood were performed. The study showed that neonatal COVID-19 was reported in eight studies, two of which were based on the detection of COVID-19 IgM in neonatal blood. Moreover, histological tests revealed sparse the virus particles, vascular mal-perfusion and inflammation in the placenta from infected mothers.[27] Shanes et al., in a study examined 16 placentas from infected mothers (15 with live birth in the 3rd trimester and one delivered in the 2nd trimester after intrauterine fetal demise). Their results revealed that third trimester placentas were significantly more likely to show at least one feature of maternal vascular malperfusion (MVM) such as abnormal or injured maternal vessels, as well as delayed villous maturation, chorangiosis, and intervillous thrombi than healthy subjects. The placenta from the infected mother with intrauterine fetal death showed villous edema and a retroplacental hematoma. However, the rates of acute and chronic inflammation were not increased. Their results suggested a systemic inflammatory or hypercoagulable state influencing placental physiology.[28]

Angiotensin-Converting Enzyme 2

Angiotensin-converting enzyme 2 (ACE2) is described as renin-angiotensin system (RAS) component and modulates blood pressure, inflammation, and fibrosis and is crucial to the pathophysiology of hypertension, cardiovascular disease, and chronic kidney disease.[29,30] ACE inhibitors are often the first-

line agents to treat the conditions in children and are among the most commonly prescribed antihypertensive medications to children.[29] ACE2 has been established as one of the main the functional host receptors for COVID-19 and may increase the risk of SARS-CoV-2 infection and COVID-19.[31,32] Like the previous SARS-CoV, it is suggested that SARS-CoV-2 binds to ACE2 to gain entry to host cells on the respiratory tract epithelium. Importantly, SARS-CoV-2 is more pathogenic, at least in part because of its 10- to 20-fold increased binding affinity to ACE2.[33,34] These findings may partially explain the easier transmissibility of SARS-CoV-2 and that raised ACE2 expression may confer increased susceptibility to host cell entry of SARS-CoV-2. However, it seems that ACE2-expressing organs do not equally participate in COVID-19 pathophysiology, implying that other mechanisms are involved in orchestrating cellular infection resulting in tissue damage.[35]

Studies demonstrated that ACE2 was highly expressed in maternal-fetal interface cells such as stromal cells and perivascular cells of decidua, and cytotrophoblast and syncytiotrophoblast in placenta.[36,37] Meng et al., collected the online available single-cell RNA sequencing (scRNA-seq) data to evaluate the cell specific expression of ACE2 in maternal-fetal interface. Their results revealed that the SARS-CoV-2 receptor was widely spread in specific cell types of maternal-fetal interface and fetal organs. Taking these functions into account, COVID-19 may disturb the female reproductive functions through regulating ACE2.[38] Moreover, the target towards the interaction between SARS-CoV-2 and ACE2 during the pandemic may be useful for treatment of the disease.[39,40]

Conclusion

A few pieces of evidence but not definitive support the possibility of trans-placental transmission of SARS-CoV-2 infection in pregnant mothers. The placentas of the infected mothers have higher rates of decidual arteriopathy and other maternal vascular malperfusion features. Practically, possible perinatal exposure such as delivery mode and time interval from delivery to the diagnosis of neonatal infection is pivotal in ascertaining congenital from perinatal infection. The paucity of placental expression of ACE-2 involved in cytoplasmic entry of COVID-19 may explain its relative insensitivity to trans-placental infection. RT-PCR tests on amniotic fluid, placenta, and cord blood are necessary to determine the possibility of trans-placental transmission of SARS-CoV-2 infection. Moreover, the in situ hybridization of COVID-19 RNA in the infected placentas increases the possibility of estimating the viral load in cells with morphological context. High quality studies are needed to further evaluate the possibility of crossing COVID-19 from placental barriers in pregnant women.

Conflict of Interests

Authors have no conflict of interests.

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Original Article

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Application of Golden Nanoparticles against *Streptococcus Mutans* for Prevention of Caries Lesions

Karim Daliri[1], Neda Seifi-Shalamzari[2*], Maryam Saeida-Ardekani[3], Sedigheh Ekraminasab[3], Hossein Neamatzadeh[3,4]

[1] Department of Medical Genetics, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
[2] Department of Emergency Medicine, Shahrekord University of Medical Science, Shahrekord, Iran
[3] Mother and Newborn Health Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[4] Department of Medical Genetics, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

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Corresponding author:
Neda Seifi-Shalamzari

Email:
seifi4145@gmail.com

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ABSTRACT

Background: Dental caries also known as baby bottle tooth decay, is a critical public health problem around the world for which *Streptococcus mutans* (*S.mutans*) has been introduced as the main infectious etiology. In the past two decades, nanotechnology has permitted the development of new materials with antimicrobial properties. The aim of this study was to compare the bactericidal and bacteriostatic effects of three golden nanoparticles (SP, NR, and CS) on *S.mutans*.

Methods: To determine the minimum inhibitory concentrations (MICs) and the minimum bactericidal concentrations (MBCs), a liquid dilution method was applied.

Results: All golden nanoparticles (GNPs) showed antimicrobial activity with no statistically significant differences (> 0.05) in MIC or MBC.

Conclusion: Our findings revealed that the size and shape of the nanoparticles did not significantly affect the antimicrobial properties of the GNPs. This finding might be useful for achieving important clinical effects with reduced toxicity in the management of early childhood caries in future in vivo studies.

Introduction

Early childhood caries (ECC) has become a major health problem, especially in the poor social population. It is characterized as the presence of one or more decayed, missing, or filled primary teeth in children aged 71 months or younger. It has several unique clinical

features, such as the rapid growth of caries, which will soon affect several teeth after appearing in the oral cavity. These lesions include dental surfaces that are less susceptible to caries development.[1,2] The etiology of ECC is multifactorial and is mostly associated with the specific interaction of microorganisms with sugars on a tooth surface. Other main causes of the ECC development include poor oral hygiene, lack of fluoride exposure, and enamel defects.[3]

Streptococcus mutans (*S.mutans*), *Streptococcus subbrinus* and Lactobacillus are the most commonly associated microorganisms in the development of caries lesions. *S.mutans* is the main pathogen in dental caries isolated from human heart valves and the blood in patients with endocarditis.[4,5] The prevalence of caries in preschool children with high levels of S.mutans in the oral cavity is higher. They also have a greater risk for development of new lesions.[6,7] *Streptococcus mutans* is usually transmitted to young children through their mothers and high maternal salivary levels of *S.mutans* increases the risk of transmission. The prevention or delay of early mutans streptococci colonization in children is associated with lower caries prevalence.[8,9]

Recent evidences have revealed increases in resistance of various pathogenic bacteria against numerous synthetic drugs. Recently, applying golden nanoparticles (GNPs) as a delivery vehicle of antibiotic agents to improve their capacity for targeting a wide range of bacteria emerged as a highly demanding topic of research in the field of nanotechnology. To overcome this problem different reports on using Au (I) and Au (III) complexes for their antimicrobial activity against a wide variety of microorganisms have released.[10,11] Moreover, different classification of GNPs are available for instance Au (I) complexes categorized into three categories based on the ligand types a) phosphine ligands b) N-heterocyclic carbene ligands c) other Au (I) complexes. The purpose for the development of gold complexes is their solubility in water and potential application as therapeutic agents.[12]

One main challenge is assessing nanoparticle toxicity to develop them for imaging, and drug delivery. It should be mentioned that if nanoparticles do not enter cells, they have less side effects such as killing cells or changing cellular function so considering dimensional limitations in designing nanoparticles for targeting cells needs more investigations on different cells. It should also take into account that gold nanoparticles have relatively good biocompatibility and low toxicity in comparison to other kinds of inorganic nanoparticles.[13,14]

Specific surface area and the reactivity of GNPs' facets affect their antibacterial activity; particles with larger effective contact area present stronger antibacterial activity.[14] Thus, gold nanowires show the weakest antibacterial activity compared with silver nanocubes and nanospheres because of their weak contact with bacteria. However, the antimicrobial activities of nanoparticle colloids of different shapes are not yet clear.[15] The purpose of this study is therefore to determine whether the size and morphology of golden nanoparticles in colloidal solution alter their antimicrobial activity. We characterized the shape and size of three different GNPs in this study [gold nanospheres, nanorods, and core/shell silica/gold nanocrystals], which were coated with monocarboxy (1-mercaptoundec-11-yl) hexaethylene glycol and their antimicrobial activity against reference stocks of *Streptococcus mutans* ATCC 25175 were analyzed as well.

Materials and Methods

AuNPs: We applied four available models of morphologically different GNPs including spherical (SP), rodlike (NR), and core/shell silica/gold (CS). All GNPs were stabilized with monocarboxy (1-mercaptoundec-11-yl) hexamethylene glycol (OEG) which improves the stability of NPs for aggregation and decreases the possibility of nonspecific

interactions with biological molecules such as serum proteins. Additionally, the negative charge of the OEG GNPs lowers their cellular toxicity. The morphology, size, and shapes of GNPs were identified using transmission electron microscopy (TEM), zeta potential measurement and spectrophotometer. TEM measurements were done on JEOL and JEM-1400 at an elevated voltage of 120 KV. A drop of colloidal gold solutions from 10 mM and 20 mM was put on TEM copper lace. After 5 minutes of drying and removing extra solution by a blotting paper, size diffusion of GNPs was measured on images of TEM. The hydrodynamic diameter of GNPs was calculated using zeta sizer nano-ZS (Malvern instrument, Malvern, UK) following the data analysis in an automated way. The UV- visible spectroscopic measurement was observed using single beam spectrophotometer (Systronics 169) at distinct wavelength (300-600 nm).

Antimicrobial tests

Strain and medium: *Streptococcus mutans* ATCC 25175 was utilized as the microbial strain which was precultivated in brain heart infusion (BHI) broth (Himedia).

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC): The minimum inhibitory concentration (MIC) was assessed utilizing a spectrophotometric microdilution method (SMM) and turbidity. Then resazurin (Sigma, St Louis, USA) as oxidation–reduction indicator to investigate viable antimicrobial activity was added. The three doses were determined for the GNPs solution. The 20 µL (low dose) solution included 1.35 µg Au NPs, 30 µL (medium dose) contained 2.03 µg Au NPs and 40 µL (high dose) contained 2.70 µg Au NPs. Four tubes of each dose were made and incubated at 37 °C for 24 h. The wells were supplied with 100 µl BHI, 100 µl test solution, and 10 µl exponentially growing bacterial culture (about 108 colony-forming units/mL). A 200 µL from each tube was distributed in each micro-well plate and seen by spectrophotometer. The optical density (OD) values from ELISA reader were used in absorption mode which covered the bacterial growth in each sample. Three values of OD for each sample and their mean were calculated with standard deviation (SD). Four tubes of each dose were made ready and incubated at 37°C for 24 h. The minimum bactericidal concentration (MBC) was discovered from the MIC value; 10 µl of the solution was pipetted from wells, located on BHI agar, and incubated at 37°C for 18 h anaerobically. For each GNP, four concentrations were examined: the MIC value, two concentrations above, and one below. The MIC values were indicated as the lowest concentration effective in suppressing bacterial growth. The MBC was defined at the point when aliquots from the MIC wells did not show visible bacterial growth on agar plates. All the experiments were done in triplicate.

Results

Characterization of nanoparticles: Figure 1 displays TEM pictures of all the different types of nanoparticles.

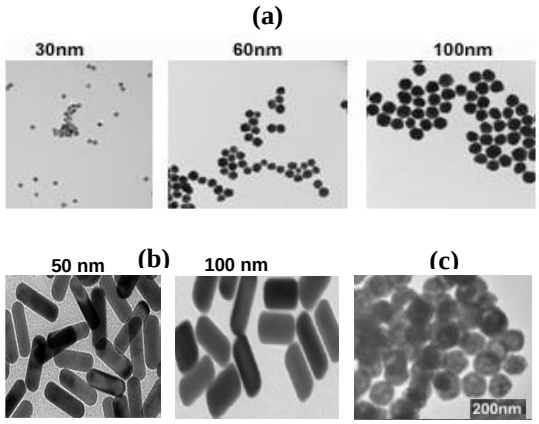

Figure 1. TEM images of gold nanoparticles: a) gold nanospheres, b) gold nanorods, c) hollow gold nanoparticles

The average size of each type AuNPs was measured by TEM images. All GNPs were stabilized with monocarboxy (1-mercaptoundec-11-yl) hexaethylene glycol (OEG). The OEG capping layer enhances the stability of NPs against aggregation and minimizes the feasibility of nonspecific

interplays with biological molecules such as serum proteins. Furthermore, the negative charge of the OEG GNPs decreases their possible cellular toxicity, whereas the ethylene glycol units may enhance the retention times of the particles in the blood. The nanoparticles were identified with TEM, and zeta potential measurements. The nanoparticles were robust in solution and did not display any signs of aggregation. Moreover, the zeta potential measurements showed a strong negative charge for all the types of OEG GNPs. The average number of OEGs attached to each nanoparticle was detected with Ellman's method. Ten independent measurements were performed for each type of NP (Table 1). The number of capping ligands was adequate to provide the appropriate surface stabilization.

Table 1. Physicochemical characterization of OEG-capped gold nanoparticles of different morphologies

OEG NSP	core (nm)	Diameter (nm)	Charge (Mv)
SP	14 ± 1	27 ± 1	-26 ± 2
NR	18 ± 1/43 ± 1	-	-29 ± 1
CS	26 ± 1/7 ± 2	45 ± 4	-31 ± 2

Antimicrobial activity of AuNPs: The bactericidal property of metals depends on the contact surface with bacterial cells, so using GNPs against *Streptococcus mutans* appears to be an alternative for stopping bacterial growth, since the large surface area of nanoparticles allows a wide range of interactions with organic and inorganic molecules. The MIC and MBC values were 3.1 ± 1.1 and 2.1 μg mL^{-1} for SP, 3.2 ± 1.7 and 4.2 μg mL^{-1} for NR, 3.4 ± 1.3, and 2.2 μg mL^{-1} for CS, respectively. The results were not significantly different (P = 0.32) for both MIC and MBC (Table 2).

Table 2. MIC and MBC of golden nanoparticles for *Streptococcus mutans* ATCC 25175

GNPs	MIC (μg mL-1)	MBC (μg mL-1)
SP	3.1 ± 1.1	3.1
NR	3.2 ± 1.7	4.2
CS	3.4 ± 1.3	3.2

Discussion

S.mutans has been considered as the principal microbiological agent in the development of dental caries. The amount of *S.mutans* naturally in the oral cavity is almost 1×10^5 colony-forming units per milliliter of saliva. Thus, a number of methods such as DNA plasmids resistant to *S.mutans*, vaccines and antibodies have been applied to reduce colonization of *S.mutans*.[16,17] Gold nanoparticles have been broadly used in bionanotechnology based on their unique properties and multiple surface functionalities for example the ease of AuNP functionalization as a versatile platform for nanobiological assemblies with antibodies, proteins and oligonucleotides. Additionally, AuNPs serve as platforms for therapeutic agents, with their large surface area allowing a dense presentation of multifunctional moieties. AuNPs have also gained attention for the design and development of innovative biomedical tools.[18] Some critical characteristics of AuNPs such as facility of functionalization, and simple synthesis allowing the release of high drug at infected sites and an ability to penetrate biological membranes which make them promising candidates for the improvement of novel antibacterial agents.[19,20] Several methods for NP surface synthesis and functionalization with antimicrobial drugs through covalent or noncovalent interactions have been described, such as amoxicillin-coated AuNPs, vancomycin-capped AuNPs, and ampicillin- and, streptomycin- conjugated AuNPs. The aforementioned studies are compatible with the application of nanoparticles combined with other antimicrobial effects to decrease intrinsic toxicity of nanoparticles, improve the microbicidal effects, and reduce the probability development of resistance.[21-23]

The current study revealed that the three GNPs presented reasonable MIC and MBC values giving a new perspective for the development of antimicrobial compounds for control of caries especially in children.

Furthermore, the results shown herein demonstrate that variation in the shape and size of the gold nanoparticles do not affect their antimicrobial ability and the color of the solution has no direct association with the antimicrobial property. Although, it is not possible to compare all the MIC/MBC values described in different investigations because of the wide range of initial bacterial concentrations, microbial strains, and culture medium components used. Antibiotics also influence the oral flora and suppress certain categories of microbes. For example, penicillin removes oral bacteria or broad-spectrum antibiotics diminish gram-positive and gram-negative bacteria, and so, provide a suitable environment for fungi and yeast to grow as opportunistic agents creating. On the other hand, the rapid spread of resistant bacteria occurring worldwide is endangering the efficacy of antibiotics, which saved millions of lives.[24]

Early childhood caries management is expensive, and often needs comprehensive treatment for the repair and extraction of teeth at an early age. As young children are not able to cope with widespread methods of treatment, deep sedation or general anesthesia may be necessary.[25] Since 1940s using fluorides to prevent dental caries, including fluoride toothpaste, water fluoridation, or professional topical fluoride application, mostly by inhibiting mineral loss from the tooth has been considered as an effective option especially in childhood caries but the multiple pathways to the development of dental caries make it difficult to ascertain the contribution of fluoride ingestion to dental caries inhibition.[26] Given that fluoride activity is effective in preventing dental caries in a topical manner so, only topical fluoride products are likely to contribute to the claimed benefits of this chemical. In addition, fluoride exposure has a complex relationship concerning dental caries and may increase the risk of dental caries in children with malnutrition due to decreased calcium and hypoplastic enamel.[27,28]

Uses of probiotics chewable tablets or supplements containing GNPs also are some feasible options in controlling the caries in children which needs more in-vivo and in vitro studies. These findings lead us to consider that SP, NR and CS materials may be useful for controlling *S.mutans* and therefore caries. Improvement of a carrier to deliver GNPs locally, as well as determining the toxicity in vivo to use GNPs as fluoride alternative in ECC management is suggested for future studies.

Conclusion

Currently, an extensive research in nanotechnology and its utilizations in infectious or contagious diseases have led to the development of antimicrobial nanoparticle formulations that act as an effective bactericidal agent. In present study, we showed the potential application of three gold nanoparticles (SP, NR, and CS) in vitro, to diminish or eradicate *S.mutans*.

Conflict of Interests

Authors have no conflict of interests.

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Original Article

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Association of +505A>G Polymorphism at TAFI Gene with Recurrent Miscarriage in Iranian Women

Razieh Alivand[1†], Fatemeh Abdi[2†], Mahmood Dehghani-Ashkezari[1*], Hossein Neamatzadeh[2,3], Sedigheh Ekraminasab[3,4]

[1] Biology Department, Medical Biotechnology Research Center, Ashkezar Branch, Islamic Azad University, Ashkezar, Yazd, Iran
[2] Department of Medical Genetics, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[3] Mother and Newborn Health Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[4] Laboratory Hematology and Blood Banking Department, School of Allied Medical Sciences, Shahid Beheshti University of Medical Science, Tehran, Iran

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† These authors contributed equally to this work.

Corresponding author:
Mahmood Dehghani-Ashkezari

Email:
mdashkezary@yahoo.com

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ABSTRACT

Background: Recurrent miscarriage (RM) is one of the major problems of public health globally. The thrombin-activatable fibrinolysis inhibitor (TAFI) gene is a plasma zymogen that regulates both fibrinolysis and inflammation. Genetic variants within TAFI gene are presumed to be associated with development of RM. This case-control study aimed to investigate the association of TAFI +505A>G polymorphism with RM in Iranian women referred to Meybod Genetic Center.

Methods: Fifty women with RM (at least 2 miscarriages) and 50 healthy women with no history of miscarriage or other fertility complications were participated in this study. The TAFI +505A>G polymorphism was genotyped by allele specific PCR (AS-PCR) assay.

Results: The mean age of cases with RM and controls was 27.25 ± 4.31 and 28.42 ± 3.22 years, respectively. The frequency of GG genotype and G allele was 0.00% in patients and controls. There was no significant difference between RM cases and controls in terms of +505A>G genotypes and alleles.

Conclusion: This study results indicated that there was no significant relationship between the TAFI +505A>G polymorphism and RM risk in Iranian women. However, further rigorous, studies with a larger sample size and different ethnicity are necessary to confirm our findings.

Introduction

Pregnancy loss is characterized as a medically identified pregnancy unintentional ending before 20 weeks or when the fetus weighs lower than 500g and increasing with maternal age.[1,2] Pregnancy loss is the most current problem of pregnancy, affecting about 10%-15% of clinically recognized pregnancies.[3] Moreover, miscarriage can be arranged as embryonic loss (or early miscarriage) when it takes place before 10 gestational weeks and fetal loss (or fetal miscarriage) when it takes place after 10 gestational weeks, because factors related to each may differ.[1,4] Recurrent pregnancy loss (RPL) is traditionally described as the event of two or more continuous pregnancy losses. RPL is one of the most common fertility complications and the exact prevalence of RPL is difficult to estimate, most studies report that RPL affects 1-5% of women during reproductive ages.[5,6] It is a multifactorial condition involving the interaction of genetic factors and environmental factors. Today several factors of RPL such as genetic thrombophilia, endocrinological factors, abnormalities in chromosomes, uterine abnormalities, thrombotic tendency, hormone or metabolic disorders have been identified. Moreover, environmental and psychological factors include infection, malefactors, autoimmunity, age, and lifestyle problems.[7,8] Also RPL can be caused by endocrine, immunological, vascular, and metabolic imbalances.[9] Although the causes have been studied deeply, more than 50% of cases remain unexplained.[3,10,11] Several articles provide evidence that genetic factors display a significant element of human fertility.[12]

Thrombophilia is one of the most reasons for RPL. Thrombophilia could be either acquired or inherited. Approximately 40% of cases displaying thrombosis are inherited. variation in the amount or the function of the proteins which are in the coagulation system pathway leads to hereditary thrombophilias.[13] Hereditary thrombophilia has been displayed to be a risk factor for reproductive diseases including infertility, RPL, and obstetrical complications.[11] The balance between coagulation and fibrinolysis is an essential part of early pregnancy.[14] At pregnancy, alterations in the mother's body due to changes in the homeostasis lead to the hyper-coagulable state of pregnancy. A hyper-coagulable state is described by exceeded levels of prothrombotic factors and reduced antithrombotic factors. Such thrombophilias enhance the prothrombotic condition of pregnancy, resulting in insufficient fetomaternal circulation, and affect the function of placentation in the developing embryo.[3] The risk of thrombophilia becomes larger which can reflect many genetic factors like polymorphisms which then affect the coagulation system. Of late, the relation of RPL with maternal thrombophilic or hypofibrinolytic gene variants has collected growing evidence. Many previous studies evaluated polymorphism of several thrombotic genes that had a role in RPL such as prothrombin 20210G/A, FVL 1691G/A, MTHFR 1298A/C, MTHFR 677C/T, and PAI-1 4G/5G polymorphisms and RPL risk in the Iranian population.[15]

Thrombin activatable fibrinolysis inhibitor (TAFI) factor, is a basic carboxypeptidase with strong antifibrinolytic and anti-inflammatory activity.[16] Practically TAFI is an unstable carboxypeptidase of plasma zymogen which forms a molecular link between coagulation and fibrinolysis.[17] The physiological function of TAFI is to dilute fibrinolysis secondary to activation of plasminogen by tPA into plasmin on the surface of a fibrin clot.[18] The proposal of most studies about TAFI is that increase in TAFI levels takes part in arterial thrombus and venous thrombus formation.[18] Also TAFI has a role in the regulation of early human trophoblastic invasion. Reportedly TAFI levels in the maternal circulation slowly increased during gestation with a peak in the last trimester, especially in complicated

pregnancy, and later come back to natural levels after the first day postpartum.[19]

In the two decades, the effects of several polymorphisms influencing TAFI level on these thrombotic events have been evaluated. Several applications such as +505A/G SNP and 1040C/T SNP in the programming region and a -438G/A SNP in promoters are associated with plasma antigen levels.[18] Also, multiple kinds of research have shown an association between thrombophilia abnormalities and their correlations to pregnancy loss. Therefore, in our study we investigated the association of TAFI +505A/G polymorphism with RPL in Iranian women.

Materials and Methods

Subjects: All procedures in the study were carried out under the ethical standards of the institutional or national research committee of Ashkzar Branch, Azad University, Ashkzar, Yazd and with the 1964 Declaration of Helsinki and its later modifications or similar ethical standards. The aims of the study were fully explained to all participants and written consent was obtained. A total of 50 women with unexplained RPL before 20 weeks' gestation were included in the study. The control group included 50 healthy women without a history of miscarriage or other fertility complications. All participants were examined by an expert gynecologist and they were checked for chromosomal abnormalities and thrombophilic factors. Both cases and healthy subjects were originally Iranian and were recruited from Meybod Genetics Center, Yazd.

DNA Isolation and Genotyping*:* Five cc of peripheral blood from peripheral blood of all subjects was collected in EDTA-containing tubes and genomic DNA was isolated using an extraction kit (Rojeh Company's Co., LTD). The quality of DNA samples was evaluated by 0.8% agarose gel electrophoresis and NanoDrop and then stored at -20C (Figure 1 and 2).

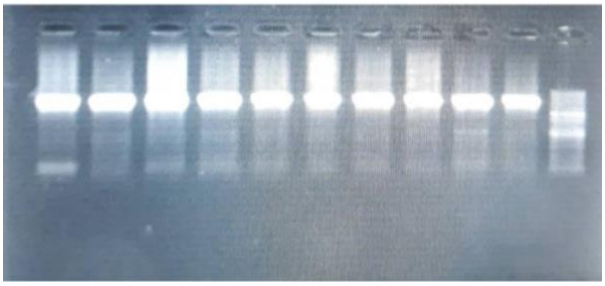

Figure 1. The quality of DNA samples was assessed by performing the 0.8% agarose gel electrophoresis

The +505A/G polymorphism at the TAFI gene was genotyped using tetra-primer amplification refractory mutation system-polymerase chain (ARMS-PCR) assay. The primers were designed using Oligo software and NCBI BLAST search engine and synthesized by Fazapjooh Tehran Company (Tehran, Iran). The size of PCR products and primer sequences for each single nucleotide polymorphism are displayed in Table 1.

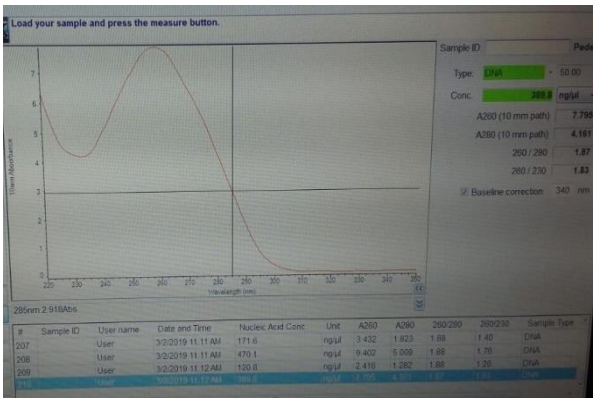

Figure 2. Assessing the quality of DNA extracted with NanoDrop

The PCR amplification was performed in a total volume of 27 µL reaction mixture, contain 5 µL genomic DNA, 10 µL Master mix 2x, 1 µL of each primer and 10 µL sterilized water. The reaction mixtures were denatured at 96°C for 5 min, followed by 36 cycles of 94°C for 50 s, 55°C for 50 s, and 72°C for 1 min, with a final elongation at 72°C for 7 min. The PCR products were separated at 37°C overnight by 1% agarose gel electrophoresis and visualized under UV light (Figure 3).

Table 1. Primer sequences for detection of TAFI +505A/G polymorphism

SNP-ID	Sequence	PCR product size (bp)
rs3742264	F-5′-CTTCCACATGCAGCTCTGAC-3′ R-5′-ATAGCCCAGTTGAGTCTGACAC-3′	180
	F-5′-GGTTTCTGGAAAAGAACTAG 3′ R-5′-CATATGGCATTTTTGGCCGT-3′	520

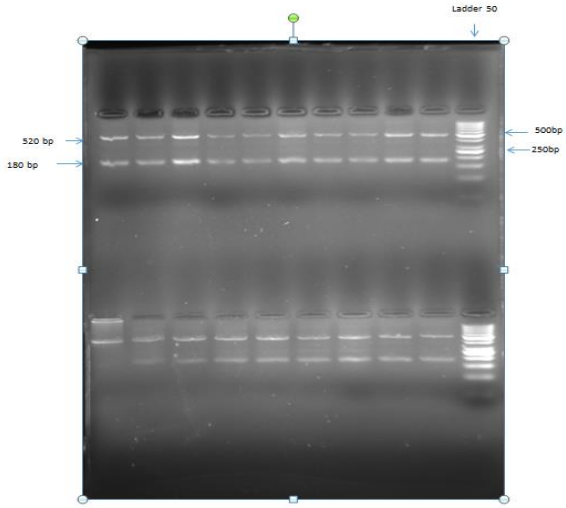

Figure 3. Scheme of electrophoresis of the PCR-RSM assay for genotyping of TAFI +505A/Gpolymorphism

Statistical analysis: All Statistical analyses were performed using SPSS version 19.0 (SPSS Co., Chicago, IL, USA) for Windows. A $P < 0.05$ was considered to demonstrate statistical significance. The chi-square test was applied to examine the differences between RPL cases and healthy controls in terms of mean age. The distribution of the genotype and allele frequency for the polymorphism between cases and controls was examined by the chi-square test. Hardy-Weinberg equilibrium (HWE) for the distributions of TAFI +505A/G genotypes was performed in healthy subjects by the chi-square (χ^2) test.

Results

Characteristics of participants: The demographic and clinical features of the participants are given in Table 2. The mean age of patients and control group was 27.25 ± 4.31 and 28.42 ± 3.22 years, respectively. Patients were divided into two groups based on the number of abortions, of which 27 patients (54%) had two abortions, 23(46%) patients had three and more than three recurrent abortions (Table 2). In the control group, they had an average of 2.34 children. In addition, in the group of patients with recurrent miscarriage, 23(46%) cases had familial marriages and 27(54%) cases had non- consanguineous marriages. Patients were also divided based on type of miscarriage. Miscarriage in 24 patients took place before 10 gestational weeks (early miscarriage) and in 26 patients took place after 10 gestational weeks (late miscarriage).

Table 2. Characteristics of cases with RPL and healthy controls

Variables	URPL Cases (n = 50)	Healthy Control (n = 50)	*P*
Age (year)			
Mean (±SD)	27.25 ± 4.31	28.42 ± 3.22	0.721
Contagious Marriage			
Familial	23	-	
Non familial	27	-	
Number of miscarriage			
2	27	-	
≥ 3	23	-	
Type of miscarriage			
Early(< 10 weeks)	24	-	
Late(> 10 weeks)	26	-	

Table 3. The frequencies of genotypes/alleles of the TAFI +505A/G polymorphism in subjects

SNPs	RPL Cases (n = 50)		Healthy Control (n = 50)		P
	Frequency	Percent	Frequency	Percent	
Genotypes					
AA	31	62%	26	52%	
AG	19	38%	24	48%	NA
GG	0	0	0	0	
Alleles					
A	81	81%	76	76%	NA
G	19	19%	24	24%	

Association between TAFI polymorphism and RPL susceptibility: Genotype and allele frequencies of TAFI +505A/G polymorphism were examined in case and control groups. Genotypes frequencies of CC for +505A/G polymorphism in women with an unexplained recurrent miscarriage were not seen. These findings showed that there was no significant difference between case and control groups. Moreover, no significant association was observed in allele frequencies and genotype distributions for +505A/G (Table 3).

Discussion

The connection of RPL with maternal thrombophilic or hypofibrinolytic gene variants has collected developing documents.[20] Such thrombophilias enhance the prothrombotic state of pregnancy, lead to insufficient fetomaternal circulation, and influence the action of placentation in the developing embryo.[20] It is recommended to investigate polymorphisms of the genes playing roles in thrombophilia in different communities so that approaches for early determination and treatment are suggested. In the two decades, several emerging candidate genes have been reported in association with RPL. One of them is the TAFI gene, which is involved in thrombosis.

A previous study evaluated polymorphism of several thrombotic genes that had a role in RPL. In 2018, Kamali et al., in a meta-analysis demonstrated that there is a significant relation between thrombotic genes including FVL 1691G/A,MTHFR 677C/T, MTHFR 1298A/C, Prothromb in 20210G/A, and PAI-1 4G/5G polymorphisms and risk of RPL in the Iranian population.[15] Chatzidimitriou et al, in a study, evaluated genetic variants at 12 thrombophilicin the Greek population. Their results indicated that ten genetic loci are mainly correlated with an augmented risk of RPL. Remarkablely, FV Leiden, FV HR2, Factor II prothrombin20210G/A, Factor XIII V34L, b-fibrinogen -455G/A, PAI-1 4G/5G, GPIIIa L33P (HPA-1a/b L33P), MTHFR 1298A/C, MTHFR 677C/T, Apo B R3500Q, ACE I/D, and Apo E2/E3/E4 have been incriminated to state mild or more severe thrombotic risks.[3]

TAFI, as markable anti-fibrinolytic factor, moderate partly the coagulation and fibrinolysis system, which is associated with an expansion incidence of venous thrombosis disorders. TAFI controls both fibrinolysis and inflammation, which both can contribute to RPL occurrence.[21]

There is no report about the association between TAFI polymorphism and miscarriage possible in Iranian women. In this study, we investigated the relationship of +505A/G polymorphism within TAFI gene with susceptibility to RPL. Our findings demonstrated that the TAFI +505A/G polymorphism was not correlated with an increased risk of RPL in Iranian women. Our findings are consistent with previous case-control studies among Spanish women. Martı´nez-Zamora et al., evaluated the decreased plasma fibrinolytic potential in cases with recurrent implantation failure after IVF and embryo transfer. Their results did not show a significant difference in the distribution of TAFI polymorphism between IVF and fertile women groups.[22] ElDanasori

et al., assessed the TAFI gene polymorphism (TAFI1040C/T) in women with RPL among Egyptian patients. They reported a higher frequency of C allele in the control group and a higher frequency of T allele in the case group with no statistical significance. Their study showed that TAFI 1040C/T could not be counted as a molecular predictive factor for RPL in Egyptians.[23] However, previous studies among European and Egyptian women showed that the polymorphism was associated with RPL risk. Masini et al., assessed the association between TAFI polymorphism and RPL among Italian women. They showed that the +505 and +1583 polymorphisms at TAFI gene were linked with RPL risk. Their results also demonstrated that SNPs directed toward increased circulating TAFI antigen levels are related to a decreased risk of RPL.[24] Nelly et al., evaluated the prevalence of VEGF, eNOS and TAFI polymorphisms among Egyptian women with RPL. They showed eNOS genetic variant associated with TAFI 1040C/T confirmed an almost one and half fold increase risk of RPL.[19] Moreover, Pruner et al., assessed R1040 C/T polymorphism in the coding region of TAFI gene and the risk of idiopathic RPL in Serbia. They recognized an enhanced frequency of R1040T/T of TAFI genotype in a patient group, recommending that this genotype could be a potential risk factor for idiopathic RPL.[21] In our study the prevalence of TAFI polymorphism was not significant and demonstrated that this polymorphism did not happen in any of the patients and control group. Therefore, well-designed epidemiological studies with a greater sample size and various subgroups are needed.

Conclusion

This study results showed that there was no obvious evidence of association between +505G/A polymorphism at TAFI gene and the risk of RPL in the Iranian women. Further rigorous designed studies with adequate sample size and in different ethnicity are needed to confirm our findings.

Conflict of Interests

Authors have no conflict of interests.

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Original Article

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A Meta-Analysis for Association of ACE I/D Polymorphism with Susceptibility to Preterm Birth

Reza Bahrami[1], Seyed Alireza Dastgheib[2*], Hossein Golestanpour[3], Elahe Akbarian[4], Alireza Emarati[4], Mohammad Jafari-Nedooshan[5], Hossein Neamatzadeh[6,7]

[1]Neonatal Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
[2]Department of Medical Genetics, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
[3]Department of Genetics, Marvdasht Branch, Azad University, Marvdasht, Iran
[4]Children Growth Disorder Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[5]Shahid Rahnamoun Hospital, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[6]Department of Medical Genetics, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[7]Mother and Newborn Health Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

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Corresponding author:
Seyed Alireza Dastgheib
Email:
dastgheibsa@gmail.com

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ABSTRACT

Background: Preterm birth is one of the main contributors to newborn mortality, morbidity, and hospitalization in the first year of life globally. To date, several numbers of studies have reported that Angiotensin-Converting enzyme Insertion/Deletion polymorphism (ACE I/D) is linked with preterm birth. But those results are conflicting. Thus, we carried out this meta-analysis to summarize the existing data and evaluated the association.

Methods: All eligible studies were collected from PubMed, Scopus, SciELO, MedRxiv, SID, China National Knowledge Infrastructure (CNKI), and Chinese Biomedical Literature Database (CBLD) up to 01 March 2021. The pooled odds ratios (ORs) and 95% confidence interval (CIs) under all five genetic models were calculated using either random-effects or fixed-effects models dependent on study heterogeneity.

Results: A total of five case-control studies with 480 preterm birth cases and 702 healthy subjects were included. Pooled data showed that the ACE I/D polymorphism was significantly associated with increased risk of preterm birth under the allele model (I vs. D: OR = 1.219, 95% CI 1.023-1.453, P = 0.027), homozygote model (II vs. DD: OR = 0.662, 95% CI 1.149-2.385, P = 0.007), and recessive model (DD vs. DI+II: OR = 0.707, 95% CI 1.082-1.948, P = 0.013). Stratified analysis by ethnicity indicated that the ACE I/D polymorphism was significantly associated with preterm birth in Caucasian descendants.

Conclusion: Our pooled data revealed that ACE I/D polymorphism is associated with the risk of preterm birth. However, larger and more rigorous studies among different populations are needed to evaluate the association with preterm birth.

Introduction

Preterm birth or preterm delivery is described as birth before 37 weeks of gestation, which remains a crucial issue in long-term morbidity and mortality in children less than 5 years of age.[1,2] More than 40% of preterm birth cases are occurred spontaneously rather than medically indicated [3, 4]. Despite advances in medicine, the rate of preterm birth is increasing globally.[5] In the United States and Europe, preterm birth occurs in 12-15% and 5-9% of pregnancies, respectively.[5-7] To date, several risk factors have been identified. However, our knowledge to predict the occurrence of preterm birth is limited.[7,8] The leading risk factor for preterm birth is a personal or family history [9]. Moreover, several lines of evidence have confirmed that genetic susceptibility is a predictor of preterm birth.[9-11] The risk of preterm delivery in mothers who have a mother or a sister with a history of preterm birth is higher than general population.[10,12] According to the previous studies in different populations the role of maternal genetic in preterm birth is between 15-40% for the maternal genetic contribution.[13] Moreover, environmental factors, as well as genetic predictors, contribute to preterm birth. This has led some to look for gene-environment interaction such as associations between candidate genes involved in metabolic detoxification and exposure to pollutants and xenobiotics (such as air pollution, sulfur dioxide, bisphenol A, agricultural pesticides, and herbicides), low choline intake during pregnancy, coffee consumption and maternal smoking.[4, 13-15]

Several molecular studies have been conducted to evaluate the association of genetic variants at progesterone receptor (PGR), Oxytocin (OXT), Oxytocin receptor (OXTR), Relaxin 2 (RLN2), follicle - stimulating hormone receptor (FSHR), insulin-like growth factor receptor (IGF1R) and prostaglandin E receptor 3 (PTGER3) genes with preterm birth.[15] Among them, the role of the angiotensin converting enzyme (ACE) gene has been evaluated by several epidemiological studies. This gene is a member of the renin-angiotensin system (RAS) or the renin-angiotensin-aldosterone system (RAAS) which is involved in catalyzing the conversion of angiotensin I into physiologically active peptide angiotensin II.[16-18] The Human ACE gene is mapped on chromosomes 17q23.3, contains 26 exons, and spans 21 kb. To date, several polymorphisms at ACE gene such as 240A > T, 2350G > A, 17888C > T and ACE I/D have been identified in association with different diseases.[19] The ACE insertion/deletion (I/D) polymorphism is a nonsense and 287 bp Alu repeat sequence of DNA in the intron 16 of ACE gene.[20-23] Some studies have examined the relation between ACE I/D polymorphism and preterm birth. But, the results are inconsistent and inconclusive that might be due to small sample size of recruited subjects. Thus, we conducted this meta-analysis by including all eligible studies to evaluate the association of ACE I/D polymorphism with preterm birth risk globally.

Materials and Methods

Publication Search: A comprehensive computer- based literature search was performed on PubMed, Web of Knowledge, Web of Science, Scopus, MedRxiv, EMBASE, Scientific Information Database (SID), WanFang, VIP, Chinese Biomedical Database (CBD), Scientific Electronic Library Online (SciELO), VIP, Chinese literature (Wan Fang), China National Knowledge Infrastructure (CNKI), China Science and Technology Journal database and Egyptian Knowledge Bank (EKB) database for finding all relevant studies on ACE I/D polymorphism and preterm birth until 01 March 2021. The following terms and keywords were used in various combinations to search: (‘’Preterm Birth’’ OR ‘’Preterm Delivery’’ OR ‘’ Spontaneous Preterm Birth’’) AND (‘’Angiotensin Converting

Enzyme'' OR ''ACE'' OR ''insertion/deletion'' OR ''I/D'' OR ''rs4646994'') AND (''Gene'' OR ''Genotype'' OR ''Allele'' OR ''Polymorphism'' OR '' Single nucleotide polymorphisms'' OR ''SNPs'' OR ''Variant'' OR ''Variation'' OR ''Mutation''). The search was carried out in English and Chinese. Moreover, the reference lists of the retrieved articles were checked to identify more potential studies missed during the online search.

Inclusion and Exclusion Criteria: The following criteria were applied for study selection: 1) case-control or cohort studies; 2) studies evaluated the association of the ACE I/D polymorphism with preterm birth risk; 3) studies with sufficient data for estimating an odds ratio (OR) with 95% confidence interval (CI). The studies with the following characteristics were excluded: 1) studies on animals; 2) case only studies; 3) studies with insufficient available data or lacking genotypes distribution data; 4) family based studies and linkage studies; 5) case reports, abstracts, letters to the editor, comments, conference abstracts, editorials, reviews, meta-analysis; and 6) published studies containing duplicate data. If there was overlapping data on the same cases included in more than one publication, only the one with the larger sample size or newly published was included in the pooling data.

Data extraction: Data was carefully extracted from all the eligible studies by two authors independently based on selection criteria. Titles and abstracts of these articles were also screened for relevance by two authors to determine which articles were to undergo full-text review. The following data were collected from each study: first author name, year of publication, country of origin, ethnicity, genotyping methods, numbers of cases and controls, genotype frequency of cases and controls, minor allele frequency (MAF) and Hardy-Weinberg equilibrium (HWE) in controls, and Newcastle-Ottawa Scale (NOS) for quality assessment of the study. If chosen articles did not report necessary data, the corresponding authors were contacted by email to request the missing data.

Assessment of study quality: The quality of the selected studies was determined by the Newcastle-Ottawa Scale (NOS). NOS has consisted of three parts including a selection of participants (four items), comparability of cases, and control groups (two items), and adequacy of Outcome (three items). It evaluated studies with a star-rating system ranging from zero to nine stars, in which the score ≥ 7 were expressed as high quality and ≤ 7 represent low or moderate quality (high or moderate risk of bias).

Statistical Analysis: The association of ACE I/D polymorphism and the preterm birth risk was evaluated by calculating the odds ratio (OR) and 95% confidence interval (95% CI). The significance of the pooled OR was evaluated by the Z-test. The pooled ORs were performed under five genetic models, i.e., allele (D vs. I), homozygote (DI vs. II), heterozygote (DD vs. II), dominant (DD+DI vs. II) and recessive model (DD vs. DI+II), respectively. A Chi-square-based Q-test was performed to evaluate the heterogeneity between these studies. The Chi-square test was used to evaluate the HWE of ACE I/D polymorphism distribution in the healthy subjects. A Cochran's Q-test was carried out to examine between- study heterogeneity and was considered significant when $P < 0.10$. Moreover, I^2 value was used for heterogeneity validation as well. The test of heterogeneity using I^2 statistics was as following: I^2 = 0-25%, no heterogeneity; I^2 = 25-50%, moderate heterogeneity; I^2=50-75%, large heterogeneity; I^2 = 75-100%, extreme heterogeneity. The pooled data in the fixed effect model (Mantel-Haenszel method) were selected when no significant between-study heterogeneity existed; otherwise, the random-effects model (DerSimonian-Laird method) was used.[24-27] A sensitivity analysis performed by the leave-one-out method to examine the effect of a single study on pooled ORs. The funnel plot

was used to assess the publication bias. The asymmetry of the funnel plot was evaluated by Egger's test. The HWE was tested by Fisher's exact test. All of the statistical calculations were performed using comprehensive meta-analysis (CMA) software version 2.0 (Biostat, USA). Two-sided $P < 0.05$ was considered statistically significant.

Results

Characteristics of the Studies: As shown in Figure 1, our initial search yielded 205 studies, and 131 were remained after removing duplicates. Following the inclusion- exclusion criteria, 126 studies were excluded. Finally, a total of five case-control studies [28-32] with 480 preterm birth cases and 702 healthy subjects were selected. Table 1 shows a summary of the characteristics of all eligible studies. The selected studies were published between 2004 and 2020.

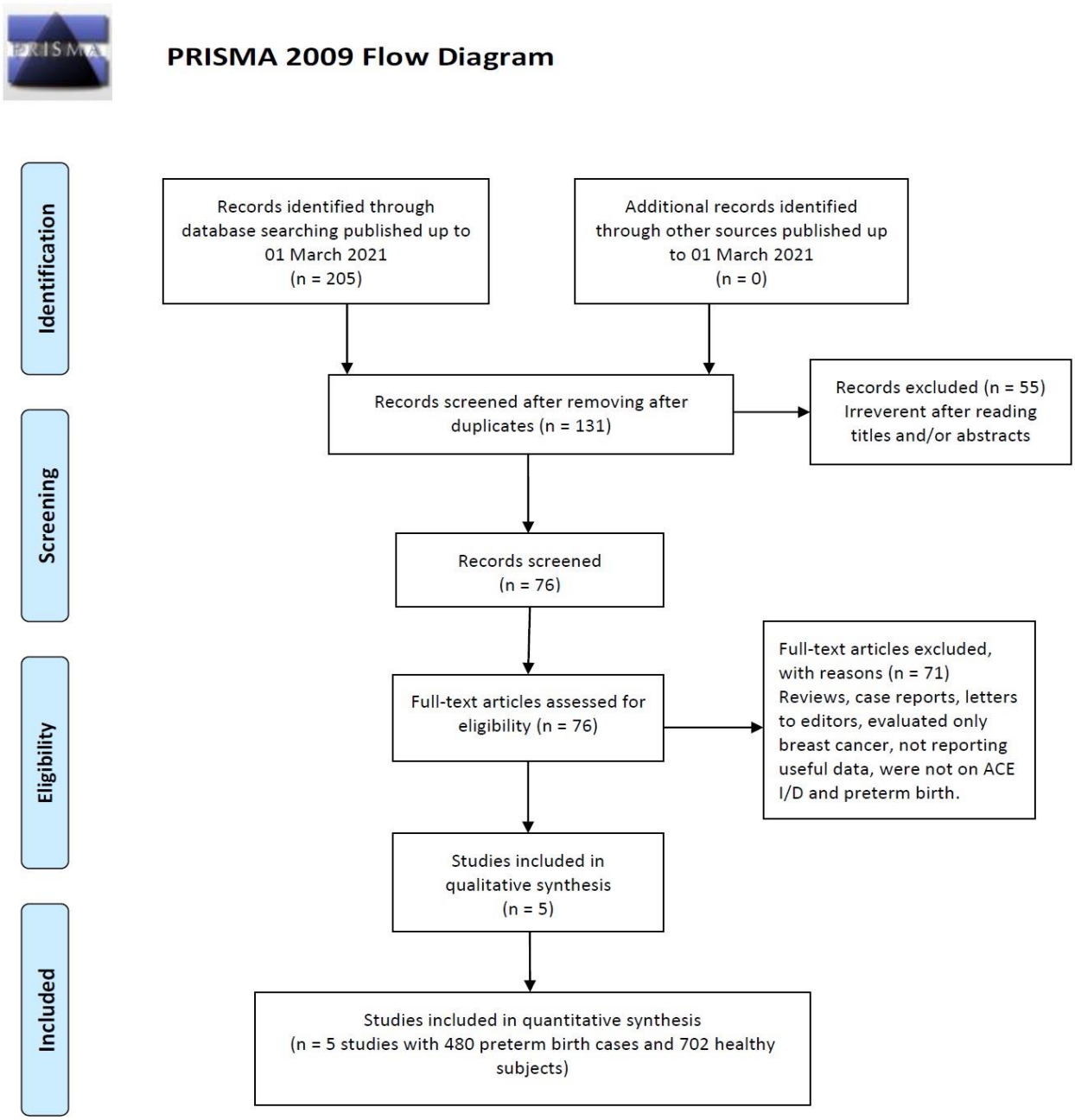

Figure 1. Flow chart for the process of selecting eligible studies

Table 1. Characteristics of the studies included in the meta-analysis

First author/Year	Country (Ethnicity)	Genotyping Methods	Case/Control	Preterm-Birth					Controls					MAFs	HWE	NOS
				Genotypes			Alleles		Genotypes			Alleles				
				II	ID	DD	I	D	II	ID	DD	I	D			
Lee 2019	Korea (Asian)	PCR	111/143	50	43	18	143	79	50	75	18	175	111	0.388	0.212	6
Hocevar 2018	Slovenia (Caucasian)	PCR	217/316	34	113	70	181	253	37	78	43	152	164	0.519	0.887	7
Uvuz 2009	Turkey (Caucasian)	PCR	50/50	15	21	14	51	49	19	26	5	64	36	0.360	0.363	6
Uma 2008	UK (Caucasian)	PCR	17/113	4	9	4	17	17	23	64	26	110	116	0.513	0.155	6
Valdez 2004	Mexico (Mixed)	Sequencing	85/238	16	44	25	76	94	66	123	49	255	221	0.464	0.548	6

MAFs: Minor Allele Frequencies; HWE: Hardy-Weinberg Equilibrium; NOS: Newcastle-Ottawa Scale

The studies have been carried out in Korea, Slovenia, turkey, England, and Mexico. In terms of ethnicity, three studies were performed among Caucasian descendants, one study among mixed population, and one study was performed among Arian descendants. Two genotyping methods including PCR and direct sequencing were used to genotype the polymorphism. The genotypes and minor allele frequency (MAF) distributions of ACE I/D polymorphism in cases and controls were presented in Table 1. Hardy-Weinberg equilibrium (HWE) was calculated for all eight publications and $P < 0.05$ was considered as a departure from HWE (Table 1). The NOS score of eligible articles ranged from 6 to 7, which showed that all included studies were of high quality (Table 1).

Quantitative Data Synthesis: The summary for the association of ACE I/D polymorphism with preterm birth risk are shown in Table 2. The combined data showed that the ACE I/D polymorphism was significantly associated with increased risk of preterm birth under the allele model (I vs. D: OR = 1.219, 95% CI 1.023-1.453, $P = 0.027$, Figure 2A), homozygote model (II vs. DD: OR = 0.662, 95% CI 1.149-2.385, $P = 0.007$, Figure 2B), and the recessive model (DD vs. DI+II: OR = 0.707, 95% CI 1.082-1.948, $P = 0.013$, Figure 2C) in overall population. Furthermore, stratified analysis by ethnicity showed that the ACE I/D polymorphism was significantly associated with preterm birth in Caucasian descendants under the allele model (I vs. D: OR = 1.316, 95% CI 1.031-1.680, $P = 0.027$) and the homozygote model (II vs. DD: OR = 1.842, 95% CI 1.109-3.060, $P = 0.018$).

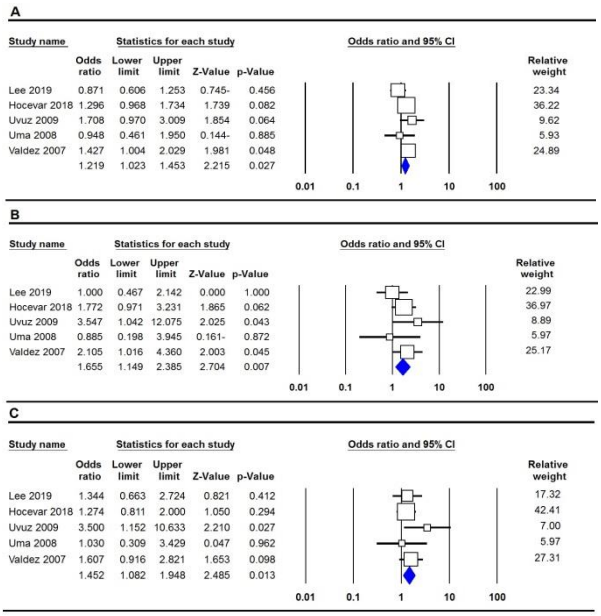

Figure 2: Forest plot for association between ACE I/D polymorphism and preterm birth risk. A: allele model (A vs. T); B: homozygote model (DD vs. II); and C: recessive model (DD vs. DI+II)

Test of heterogeneity: The heterogeneity in overall population and by stratified analyses is presented in Table 2. In this study, there was no significant between-study heterogeneity under all five genetic models in the overall population. Therefore, the fixed effect model (Mantel-Haenszel method) was selected to report the ORs for the association.

Table 2. Summary risk estimates for association of the ACE I/D polymorphism with preterm birth risk

Subgroup	Genetic Model	Type of Model	Heterogeneity		Odds Ratio(OR)				Publication Bias	
			I^2 (%)	P_H	OR	95% CI	Z_{OR}	P_{OR}	P_{Beggs}	P_{Eggers}
Overall	D vs. I	Fixed	33.94	0.196	1.219	1.023-1.453	2.215	0.027	1.000	0.960
	DI vs. II	Fixed	50.77	0.087	1.042	0.771-1.409	0.270	0.787	0.806	0.958
	DD vs. II	Fixed	7.158	0.366	1.655	1.149-2.385	2.704	0.007	1.000	0.971
	DD+DI vs. II	Fixed	52.41	0.078	1.170	0.880-1.555	1.082	0.279	0.806	0.943
	DD vs. DI+II	Fixed	0.00	0.522	1.452	1.082-1.948	2.485	0.013	0.806	0.495
Ethnicity										
Caucasian	D vs. I	Fixed	0.00	0.445	1.316	1.031-1.680	2.205	0.027	1.000	0.928
	DI vs. II	Fixed	0.00	0.524	1.311	0.846-2.031	1.213	0.225	0.296	0.117
	DD vs. II	Fixed	1.920	0.361	1.842	1.109-3.060	2.360	0.018	0.311	0.211
	DD+DI vs. II	Fixed	0.00	0.594	1.467	0.970-2.220	1.816	0.069	0.202	0.296
	DD vs. DI+II	Fixed	33.97	0.220	1.414	0.953-2.099	1.720	0.085	1.000	0.683

NA: Not Applicable

Sensitivity analysis: A sensitivity analysis is necessary to explore the impact of different decisions on pooled ORs. We carried out a sensitivity analysis to assess the effect of individual study by excluding a single study in turn on pooled data. The results showed that no individual study had an influence on the pooled OR for association of ACE I/D polymorphism with preterm birth, suggesting the stability of our conclusions.

Publication bias: Begg's funnel plot and Egger's test were used to assess the potential publication bias for included studies on ACE I/D polymorphism with preterm birth. The Egger's test results under all five genetic models are presented in Table 2. The Egger's test and Begg's funnel did not statistically revealed a significant publication bias in any of the models for ACE I/D polymorphism association with preterm birth (Figure 3).

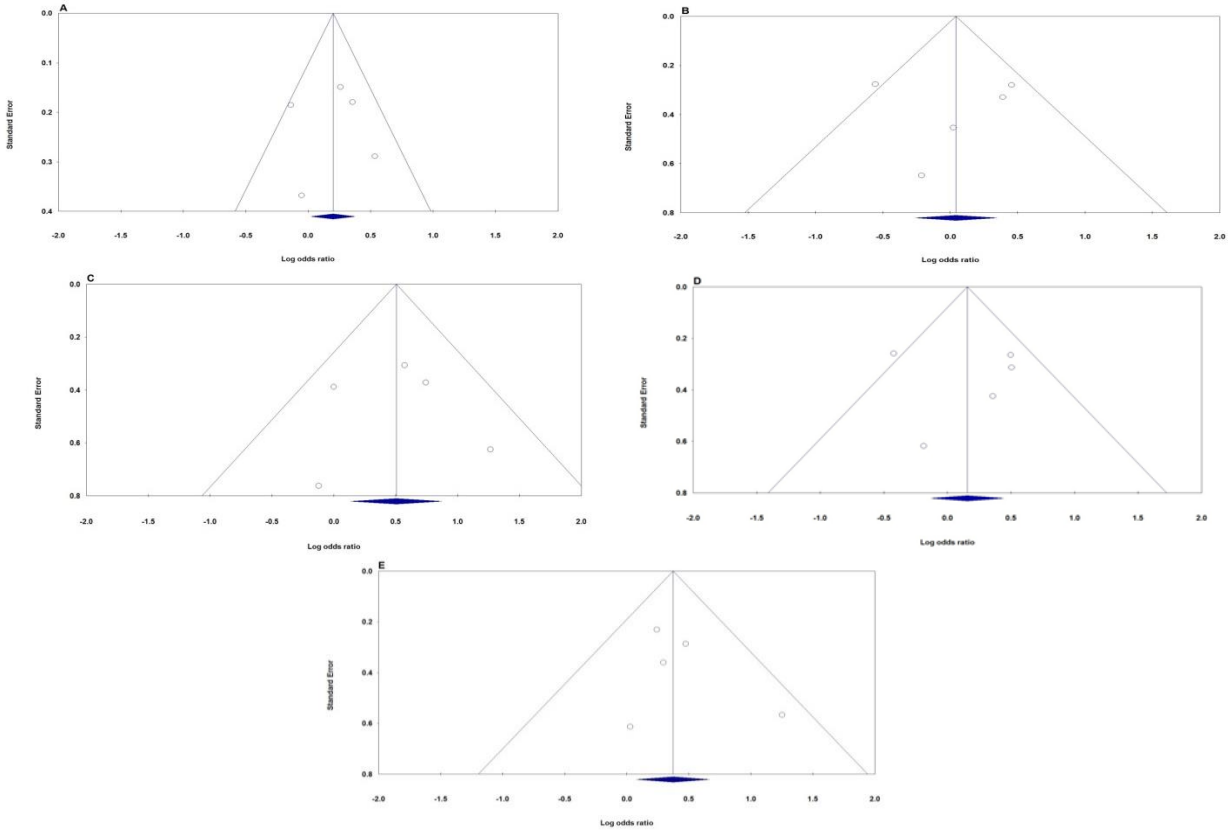

Figure 3: The funnel plots of publication bias for association of ACE I/D polymorphism and preterm birth risk. A: allele (D vs. I); B: homozygote (DI vs. II); C: heterozygote (DD vs. II); D: dominant (DD+DI vs. II) and E: recessive model (DD vs. DI+II)

Discussion

In 2004, Valdez et al., for first time assessed the association of ACE I/D polymorphism with preterm birth risk. They have evaluated the association in 86 women with preterm birth and a control group of adults from Guadalajara, Mexico. The study reported significant differences in the frequency of ACE I/D between women who had a history of preterm birth and healthy subjects.[32] Since then, a few studies evaluated the association of ACE I/D polymorphism with preterm birth risk in limited populations.

Here, we carried out a meta-analysis to assess the association of the ACE I/D polymorphisms with preterm birth risk. In meta-analysis a total of five case-control studies with 480 preterm birth cases and 702 healthy subjects were included. The combined data showed that the ACE I/D polymorphism was significantly associated with increased risk of preterm birth under the allele model (I vs. D: OR = 1.219, 95% CI 1.023-1.453, *P* = 0.027), homozygote model (II vs. DD: OR = 0.662, 95% CI 1.149-2.385, *P* = 0.007), and the heterozygote model (ID vs. DD: OR = 0.707, 95% CI 1.082-1.948, *P* = 0.013) in overall population. Lee et al., in a case-control study and meta-analysis evaluated the association of the ACE I/D polymorphism with preterm birth risk. They evaluated 111 patients with preterm birth and 143 women at ≥ 38 week's gestation as controls in the Korean population. Their case-control study revealed that the ACE I/D polymorphism was significantly associated with preterm birth and that the ID genotype of ACE I/D polymorphism has a protective effect for preterm birth. Similarly, their pooled data revealed that the ACE ID genotype has a significant association with preterm birth and is a protective factor for the disease.[28] Moreover, Hočevar et al., in another case-control study and meta-analysis evaluated the association of the ACE I/D polymorphism with preterm birth risk. Their case-control study included 217 women with a history of preterm birth and 158 women with full-term pregnancy in Serbian population. Their case-control study did not show a significant association of ACE I/D polymorphism with preterm birth. However, their pooled ORs indicated that ACE I/D polymorphism was associated with preterm birth under three genetic models including allele (D vs. I: OR = 1.35, 95% CI = 1.11-1.65, *P* = 0.0033), dominant (DD + ID vs. II: OR = 1.52, 95% CI = 1.08-2.15, *P* = 0.0161) and recessive (DD vs. ID + II: OR = 1.48, 95% CI = 1.07-2.04, *P* = 0.0184).[29]

Some limitations of this meta-analysis should be considered. First, the number of included studies to evaluate the association of ACE I/D polymorphism with risk of preterm birth was not large enough to generate meaningful results, which pooled results based on restricted studies that lack sufficient power to support or deny an association. Second, in this meta-analysis, there were included limited studies by ethnicity. Thus, the discrepancy of the associations in different ethnicities should be interpreted cautiously. Third, the strength of the association was measured by unadjusted ORs for confounding factors such as age, gestational age, and environmental factors due to the lack of primary data, which might have affected our results. Finally, preterm birth is a multifactorial disease and interactions between genetic and environmental factors might affect the development of this disease. In the current meta-analysis, gene-gene and gene-environment interactions were not evaluated due to the limited availability of such data.

Conclusion

Considering all the findings, this meta-analysis indicated that the ACE I/D polymorphism is associated with an increased risk of preterm birth in overall population. Our pooled data may help understand the role and mechanism of the ACE gene in development of preterm birth. However, larger and more rigorous studies among

different ethnicities are needed to evaluate the association of ACE I/D polymorphism with preterm birth.

Conflict of Interests

Authors have no conflict of interests.

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Immune and Non-Immune Etiology of Thrombocytopenia: Neonatal and Maternal Causes

Mohamad Hosein Lookzadeh[1], Seyed Reza Mirjalili[1], Sedigheh Ekraminasab[1,2*]

[1] Mother and Newborn Health Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[2] Laboratory Hematology and Blood Banking Department, School of Allied Medical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran

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Corresponding author:
Sedigheh Ekraminasab

Email:
s.ekraminasab@gmail.com

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ABSTRACT

Neonatal thrombocytopenia (NT) is a common hemostatic abnormalitiy among newborn in the NICU, which increases with the degree of prematurity. It is well documented that this disease has a large range of feasible etiologies. Prematurity, early and late-onset sepsis and asphyxia are the most usual causes of NT. Moreover, FNAIT is the major risk for intracranial hemorrhage in the fetus or newborn. Here, we reviewed the causes for NT, in both newborns and mothers. We demonstrated the factors associated with NT in the newborn including placental insufficiency, fetal and neonatal alloimmune thrombocytopenia (FNAIT), prematurity, sepsis, and asphyxia. The causes of thrombocytopenia in pregnant women and its impact on newborns were also described. This review showed that gestational thrombocytopenia was the most common cause of thrombocytopenia with an incidence of 70-80%, followed by preeclampsia, HELLP and ITP. But neonates born to mothers with immune thrombocytopenia (ITP) had a higher risk for NT and hemorrhagic problems. In ITP, neonatal platelets are destroyed by maternal autoantibodies. We reviewed the causes of thrombocytopenia in neonates and mothers in two groups of immune and nonimmune factors. However, it seems that immunological factors are the most severe form of NT. However, it is necessary to separate NT etiology for differential diagnosis.

Introduction

Thrombocytopenia is a condition that platelet count less than 150×10^3/L. it is one of the most common hemostatic abnormalities among newborns, particularly premature infants.[1,2] It affects 18-35% of neonates referred into neonatal intensive care units (NICU) and may lead to a high risk of hemorrhage and fatality.[2,3] Many studies demonstrated that the possibility of enhancing thrombocytopenia increases with the level of prematurity, that immature neonates were at a 2.52-fold increased risk for thrombocytopenia.[4] The etiology of thrombocytopenia is complex and both maternal and newborn factors may be implied in the development of it. Generally, Thrombocytopenia may be the only clinical apparition of alloimmune condition or an expression of other diseases, such as intrauterine growth restriction (IUGR), sepsis, or necrotizing enterocolitis (NEC). NEC is inflammatory bowel necrosis of premature infants.[5,6]

Neonatal thrombocytopenia (NT) is a common clinical issue, which enhances with the degree of prematurity. The basic causes of NT are now becoming distinct and many opinions have newly been shown to have small or no evidence to support them. We described thrombocytopenia in neonate and its etiology. Previous studies demonstrated that prematurity, placental insufficiency, sepsis, abnormal immunity, and asphyxia were the most common neonatal conditions related with NT.[7] In an otherwise healthy-appearing infant, thrombocytopenia is most probably secondary to placental inadequacy or an immune process, either autoimmune or alloimmune, in which maternal antibodies transmitted to the newborn in-utero cause the destruction of the infant's platelets.[8]

Several studies have evaluated the prevalence of thrombocytopenia during pregnancy, its etiology, and maternal and perinatal outcome. Pregnancy thrombocytopenia is a usual finding and occurs approximately in 7-10% of pregnancies. Gestational thrombocytopenia (GT), hypertensive disorders (preeclampsia; eclampsia; HELLP syndrome; and acute fatty liver of pregnancy), disseminated intravascular coagulation (DIC), consumption of drugs and vitamin B12 or folate deficiency, are the nonimmune reasons.[9,10] Overall, about 3-4% of pregnancy thrombocytopenia is related to an immune process include TTP and ITP. Immune thrombocytopenia (ITP) is an autoimmune disorder described by low platelet counts that occurs as a result of maternal autoantibodies transportation through the placenta and target platelet membrane so neonatal platelets are destroyed.[11]

Determining the causes and mechanisms of NT is the best way to develop the more appropriate treatment, including modern approaches. For example detection of NT's main causes is important to identify neonates at risk of hemorrhage and select who would benefit from platelet transfusion (PT) and to determine whether PT either abolish or intensify common neonatal problems such as sepsis, chronic lung disease, NEC, and retinopathy of prematurity.[12] In This paper we explain prevalent opinion about the reasons of NT considering fetomaternal and neonatal conditions and causes of thrombocytopenia in immune and nonimmune factors.

Causes and Mechanisms

Neonatal diseases: Almost 9.4-35% of neonates admitted to NICUs develop thrombocytopenia. Multiple disease processes can result in thrombocytopenia in neonates and these can be arranged as early-onset (< 72 hours) and late-onset (> 72 hours) NT. The significant causes of thrombocytopenia in neonates are low birth weight, sepsis, prematurity, birth asphyxia, intrauterine growth retardation, hyperbilirubinemia, and meconium aspiration syndrome. Apart from platelet counts, bleeding disorders depend on underlying diseases.[13]

Table 1. Causes of neonatal thrombocytopenias: Neonatal and maternal diseases

			Prevalence
Neonatal	Nonimmune	Placental insufficiency	-
		Perinatal asphyxia	-
		Perinatal infection	-
		DIC	-
	Immune	FNAIT	-
Maternal (pregnancy-associated causes)	Nonimmune	Gestational thrombocytopenia	70-80%
		Preeclampsia	15–22%
		HELLP	1-4 %
		Acute fatty liver of pregnancy	< 1%
		Dengue	1-2%
	Immune	ITP	2%

Apparently neonates with thrombocytopenia may develop a high risk of hemorrhage and fatality. This increased risk is associated with the important role of platelets in the whole process of hemostasis, and thrombocytopenia may lead to dysfunctional hemostasis. The differential diagnosis for thrombocytopenia is traditionally divided into disorders of decreased platelet production against those of increased platelet consumption or destruction. We arranged causes of thrombocytopenia in immune and nonimmune reasons. Decreased platelet production and increased platelet consumption (sepsis, placental insufficiency, and birth asphyxia) are the nonimmune reason, and destruction with antibodies is classified as the immune reason of thrombocytopenia (Table 1, 2).

Table 2. Comparison of early and late onset of thrombocytopenia in premature neonates

Early onset	Sepsis
< 24 hours	TORCH infection
	Birth asphyxia
	DIC
	NEC
Late onset	Sepsis
> 72 hours	Thrombosis
	DIC
	NEC
	Drug-induced

Nonimmune

Decreased platelet production: Hematopoietic stem cells (HSCs) are pluripotent cells that inhabit in the bone marrow (BM) and can differentiate into all blood cell lineages.[14] Platelet production defined as thrombopoiesis, is a convened process that results in the manufacture of thrombopoietin (TPO) as the thrombopoietic stimulus leading to the proliferation of megakaryocyte progenitors.[12,15] Megakaryocytes (MKs) in the BM generate blood platelets, necessary for thrombosis and hemostasis. MKs derive from HSCs and migrate from an endosteal niche towards the vascular sinusoids during their maturation.[16] TPO is the hematopoietic growth factor and is the major cytokine triggering platelet production. TPO contributes to the self-renewal of HSCs and also persuades transcription factors causing to the expression of proteins like CD42 or CD41 that commit HSCs to the platelet lineage.[17]

Situations increased platelet consumption

Sepsis: Sepsis is one of the major reasons of thrombocytopenia in neonates. Thrombocytopenia is presented in neonates with bacterial, fungal, viral, rickettsial and, protozoal infections. Thrombocytopenia in the very premature infant is most often secondary to sepsis, come after NEC, birth asphyxia, chronic intrauterine hypoxia, DIC and TORCH (toxoplasmosis, other agents, rubella, cytomegalovirus, and herpes simplex) infections.[18,19] Bacterial infections can be related to thrombocytopenia or reactive thrombocytosis. The pathogenic actions complicated in thrombocytopenia are numerous and include the incidence of DIC

during sepsis, the adhesion of platelets to the activated vascular endothelium, or an increased consumption related with the formation of neutrophil extracellular catchs.[19,20] Several patients with bacterial septicemia may extend coagulopathy related to DIC. The presence of thrombocytopenia is seen mostly in early sepsis with or without laboratory evidence of obvious DIC.[21] Thrombocytopenia may quickly become very serious with the lowest platelet count extended within 24-48 hours after beginning of contamination. The significance of the relation between thrombocytopenia and sepsis was confirmed by recognizing thrombocytopenia as one of the most prognosticate and autonomous risk factors for sepsis-associated fatality in very low birth weight neonates.[7]

Placental abruption: Placental abruption, defined as the premature separation of the normally implanted placenta from the uterus, before birth and after 20 weeks of pregnancy. Placental abruption is one of the most considerable determinants of maternal morbidity as well as perinatal morbidity and fatality and complicates approximately 1% of births.[22] The incidence of concomitant DIC can cause a range of problems to both mother and neonate like as emergency cesarean transfer due to unreliable fetal condition, cerebral palsy, acute hemorrhage, uncontrollable hemorrhage requiring hysterectomy, multi-organ defeat, and maternal and fetal death.[23] Thus, placental abruption is a situation needing proper perinatal management. While primary recognition and quick cure are both necessary for making the outcomes better for mother and neonate, high-level medical facilities and improvement of the emergency patient transport system are also important.[24]

Immune

Fetal and neonatal alloimmune thrombocytopenia: Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is a disorder in pregnant women. The incidence of FNAIT is approximately 1 in 1000 pregnancies.[25,26] Differences in platelet type between the fetus and the mother can result in maternal immunization and destruction of the fetal platelets, specified by maternal alloantibodies directed against the human platelet antigen (HPA). When these HPA-alloantibodies get into the fetal circulation after entering the placenta via FcRn transport, they can destruct fetal platelets as well as damage endothelial cells, which may cause haemorrhagic problems. These antibodies can lead to intracranial hemorrhage (ICH) or other great bleeding resulting in long-lasting defects or death.[27] Optimal fetal care can be provided by at the right time identification of pregnancies at risk.[28] These bleedings can alter from small skin appearances to severe ICHs or even perinatal death. In lack of population-based screening for FNAIT, cases are mostly diagnosed in case of manifestation. Consequently, FNAIT requires fast identification and therapy; following pregnancies need close monitoring and management.[29,30]

Causes of Neonatal Thrombocytopenia: maternal diseases

Thrombocytopenia during Pregnancy: Thrombocytopenia is second commonest hematological disorder in pregnancy after anemia,[9] that affects approximately 7-10% of pregnancy.[31] Most studies report a reduction in platelet count about 10% lower than the pre-pregnant values. During normal pregnancy, there is a physiological reduction in platelet count due to hemodilution, increased consumption in peripheral tissue and increased aggregation. The causes include gestational thrombocytopenia (GT) and hypertensive disorders (preeclampsia; eclampsia; HELLP syndrome; and acute fatty liver of pregnancy (AFLP)) are nonimmune. Other nonimmune causes are DIC, hemolytic uremic syndrome, consumption of drugs, vitamin B12 or folate deficiency, aplastic anemia, myelophthisis and viral infections. The most common immune diseases include

immune thrombocytopenic purpura (ITP), thrombotic thrombocytopenic purpura (TTP), autoimmune disorders, and pseudothrombocytopenia can result from ethylenediaminetetraacetic acid (EDTA)-induced clumping of platelets, in which case, a new sample should be analyzed using citrate as an anticoagulant.[32] Thrombocytopenia during pregnancy might also give a biomarker of a coexisting systemic or gestational problems and a potential cause for a maternal intervention or treatment that possible induce hurt to the fetus.[33] It can be a diagnostic and management problem, and has numerous causes, some of this are specific to pregnancy.

Non-immune

Gestational thrombocytopenia: Gestational thrombocytopenia (GT) is the most common reason of thrombocytopenia during pregnancy and associated with moderate thrombocytopenia. GT take place in 4.4% to 11.6% of pregnancies, reporting for about 75% of all cases of thrombocytopenia in pregnancy. Platelet count < 70,000/μL excludes the diagnosis of GT. GT happens in 3rd trimester of pregnancy that is most possible from hemodilution related to an increase in plasma volume during pregnancy and possibly increased platelet clearance as mean platelet volumes, and platelet-derived cyclooxygenase products rise. Thrombocytopenia is more frequent in twin and triplet gestations. The pathophysiology entails that the fetus waste products into mothers blood increases the activity of spleen of mother which removes blood cells rapidly causing destruction of platelets. Patients generally show no alarming symptom due to GT. A subset of women with GT develop a more significant decrease in platelet count and a reduction in antithrombin III, recommending a distinct pathogenesis that depends on a sequence with the HELLP syndrome and AFLP and that may be correlated with a higher risk of relapse in subsequent pregnancies. GT needs no treatment but resolves spontaneously. The diagnosis of GT is challenging and is difficult to differentiate between ITP and GT.[34]

Hypertensive disorders: Hypertensive disorders were the 2nd most common cause of thrombocytopenia during pregnancy (15- 20%). hypertensive disorders include preeclampsia, eclampsia, HELLP syndrome, acute fatty liver of pregnancy.[35] The pathophysiologic mechanism of thrombocytopenia in hypertensive disorders is the thrombotic microangiopathy distinguish by endothelial hurt, after platelet aggregation and then formation of thrombus in small vessels. The indications of thrombotic microangiopathy are the existence of schistocytes on peripheral blood smear and increased bilirubin > 1.2 mg/ dL, reduced haptoglobin < 25 mg/dl and increased LDH.

Immune thrombocytopenia

ITP: Immune thrombocytopenic purpura (ITP), an autoimmune disease determined by the anti-platelet glycoprotein (GP) antibodies that induce the platelet destruction in the spleen, is a uncommon cause of thrombocytopenia in pregnancy. However ITP takes place only in 3- 4% of all patients of thrombocytopenia during pregnancy, it is the most current reason of a platelet count below 50×10^3 per μL indicate in the first and second trimesters. Platelet counts may reduce during pregnancy, and at least 15% to 35% of mothers need treatment even prior to management of labor and delivery. This form relies on operation patterns, so that require for treatment is probably to be more current in tertiary-care referral centers. Maternal and neonatal outcomes are commonly good.[33] differently from GT, ITP can happen anytime during gestation and almost all gravid women with ITP may have a history of thrombocytopenia previous to pregnancy. The platelet count does not automatically cure postpartum and the therapeutic reply to steroids or intravenous immunoglobulin (IVIg) contributes to the treatment of ITP. In

conclusion, a neonatal outcome with maternal ITP is generally good.

Discussion

Neonatal thrombocytopenia (NT) is one of the most common hemostatic abnormalities among newborns in the NICU and overrepresented among extremely low birth weight neonates.[36] NT has many potential etiologies and a wide range of diseases have a role in its occurrence.[6] So many studies have described different aspects of NT include causes, prevalence, clinical lab diagnostics, risk factors, risk category, time of onset, bleeding manifestation, treatment, and platelet transfusion.[37,38] There is a need to arrange and separate the etiology of it, so we evaluated neonatal and maternal causes together and described several etiologies.

The most common factors associated with NT in the newborn include placental abruption, placental insufficiency (intrauterine growth restriction (IUGR)), fetal and neonatal alloimmune thrombocytopenia (FNAIT), prematurity, sepsis (bacterial, fungal, and viral), NEC, asphyxia, meconium aspiration syndrome, DIC, hyperbilirubinemia, and anemia. The previous studies showed prematurity and sepsis and asphyxia were the most usual causes of NT.[2,12,13,37]

But, FNAIT is the serious risk for intracranial hemorrhage in the infant. Thrombocytopenia in a very immature newborn is most recurrently secondary to sepsis, subsequent by necrotizing enterocolitis (NEC), birth asphyxia, chronic intrauterine hypoxia, TORCH infections, or DIC.[39]

In this topic, we also characterized determining reasons of thrombocytopenia in pregnant women and its impact on newborns. The most current factors associated with NT in pregnancy include gestational thrombocytopenia (GT) and hypertensive disorders (preeclampsia; eclampsia; HELLP syndrome; and acute fatty liver of pregnancy (AFLP)) are nonimmune.[40] Other nonimmune causes are thrombotic thrombocytopenic purpura (TTP), DIC, hemolytic uremic syndrome, consumption of drugs, vitamin B12 or folate deficiency, aplastic anemia, leukemia, systemic lupus erythematosus (SLE) myelophthisis, and viral infections.[32] Gestational thrombocytopenia was the commonest cause of thrombocytopenia with an incidence of 70-80%, followed by preeclampsia, HELLP, and ITP.[41] GT is associated with better fetomaternal outcomes compared with other etiologies.[32] ITP diagnosed before or during pregnancy is important for both the mother and the newborn.[42] The risk of intense thrombocytopenia at delivery is more in ITP contrast with chronic ITP. Patients with GT and ITP have better maternal and perinatal outcomes as compared to hypertensive disorders include preeclampsia and HELLP syndrome.[43]

We also separated etiology in immune and nonimmune reasons. Decreased platelet production and increased platelet consumption (sepsis, placental insufficiency, and birth asphyxia) are the nonimmune reason, and destruction with antibodies is classified as the immune reason of thrombocytopenia. The most common immune diseases that have a role in NT are FNAIT and ITP. In FNAIT maternal antibodies destroyed fetal platelets that lead to hemorrhagic problems intracranial hemorrhage (ICH) or other great bleeding resulting in long-lasting defects or death.[25] Optimal fetal care can be provided by at the right time identification of pregnancies at risk. These bleedings can alter from small skin appearances to severe ICHs or even perinatal death.[38] Consequently, FNAIT requires fast identification and therapy. In ITP neonatal platelets are destructed by maternal autoantibodies. Also, in ITP is widely accepted that the frequency of intracranial hemorrhage is very rare.[44] Generally in ITP maternal and neonatal outcomes are acceptable. But in ITP The platelet count does not automatically cure postpartum and the therapeutic reply to steroids or Intravenous Immunoglobulin (IVIg) is needed.[45]

Best choice for treatment of NT according to the exact cause may be useful for clinicians. Detection of neonatal thrombocytopenia's main causes is important to identify neonates at risk of bleeding and select who would benefit from PT and to determine whether PT either abrogates or exacerbate common neonatal complications such as sepsis, chronic lung disease, necrotizing enterocolitis (NEC), and retinopathy of prematurity.[12,46] Identification is largely based on the timing of its beginning, intensity of the thrombocytopenia, and the dependent with other disorders.[34,47] There is a need for another study to compare risk factors between the neonatal and maternal group and differential diagnosis, and evaluated some other possible etiology.

Conclusion

In our study, we described different immunologic and nonimmunologic causes of neonatal thrombocytopenia. We also explained the most neonatal and maternal diseases that have roles in NT. However nonimmunologic causes are most prevalent but it seems immunological disorder is more severe than other causes. For example, several studies displayed that prematurity, sepsis, and asphyxia were the most usual factors related with NT. But in neonatal causes, FNAIT leads to hemorrhagic problems, intracranial hemorrhage (ICH), or other great bleeding resulting in long-lasting defects or death. In maternal reasons GT was the most current reason of thrombocytopenia with an incidence of 70-80%, but, it is associated with better fetomaternal outcomes compared with other etiologies. Also, thrombocytopenia is solitarily related to maternal hypertension, sepsis, and intravascular thrombosis. Optimal care of thrombocytopenia depends on the right time identification of pregnancies at risk and management etiology of it. Therefore monitoring of platelet count in pregnant women should be a routine preventive exam. The clinician should be aware of differential diagnosis findings and associated with unusual causes of thrombocytopenia that should prompt additional evaluation in the NICU.

Conflict of Interests

Authors have no conflict of interests.

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Case Report

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A Case Report of Hyperphosphatasia Treated with Pamidronate

Naser Ali Mirhosseini[1*], Sana Taghiyar[2], Mahdieh Saatchi[1]

[1] Department of Pediatrics, Shahid Sadoughi Hospital, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[2] Department of Clinical Biochemistry, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

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Corresponding author:
Naser Ali Mirhosseini

Email:
nasserali.mirhosseini@yahoo.com

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ABSTRACT

Background: Hereditary hyperphosphatasia is a congenital and rare disease with high bone turn over. The disease is defined with extremely elevated alkaline phosphatase levels. Neonates with hyperphosphatasia are normal at birth but develop progressive long bone deformities, fracture, vertebral collapse, skull enlargement due to massively thickened calvarium, and deafness.

Case Presentation: Here, we described a male patient with progressive deformity in limbs and pain during walking that onset of symptoms was from age of two. The patient admitted to the Shahid Sadoughi Hospital, Yazd, was born from a non-consanguineous marriage. He was treated with pamidronate until halt of the disease progression and followed up for 18 months.

Conclusion: Bisphosphonate is the treatment of choice for hyperphosphatasia because it can normalize bone turnover, improve growth rates, and skeletal quality.

Introduction

Hereditary hyperphosphatasia is a rare high bone turn over and autosomal recessive bone disease with extremely elevated alkaline phosphatase levels and normal calcium and phosphate levels which occurs in infancy or early childhood with male predilection.[1,2] Families have been characterized as having a homozygous deletion of the *TNFRSF11B* gene that encodes osteoprotegerin (OPG).[3] The loss of OPG function results in generalized extremely rapid bone turnover.[4] Bone turnover is high and its markers such as alkaline phosphatase and hydroxyproline are increased.[5]

The phenotypic variability ranges from presentation in infancy with severe progressive deformity to presentation in late childhood with minimal deformity.[6] Osteoid proliferation in the subperiosteal portion of

bone leads to separation of the periosteum from the bone cortex. Bowing and thickening of the diaphysis are common along with osteopenia. Affected children are normal at birth but the disease usually has its onset by 2-3 years of age when painful deformity developing in the extremities results in abnormal gait and sometimes fractures. Other common findings include pectus excavatum, kyphoscoliosis, and rib fraying. The skull is long and the cranium is thickened and may be deformed. Progressive and profound hearing loss can arise because of skull involvement.[7]

On radiographs, the bony texture is variable, dense area interspersed with radiolucent areas and general demineralization (cotton- wool appearance). Long bones appear cylindrical, lose metaphyseal modeling, and contain pseudocysts that show a dense, bony halo.[7]

At present, treatment is far from optimal and consists of calcitonin to reduce bone turn over and bisphosphonates to inhibit bone resorption. Therapy with recombinant osteoprotegerin has shown promising results in adults.[6] Intravenous pamidronate therapy can reduce the rapid bone turnover, bone pain, prevent deformity and disability and improve hearing. However, this effect can be transient. In case of resistance to pamidronate therapy, switching to another bisphosphonate like zoledronate may provide long-term clinical and biochemical improvement as an alternative treatment.[5] Cyclical intravenous pamidronate (1 mg/kg/day during 3 h, 3 consecutive days at 2- to 3-month intervals) administration for 2 years with oral calcium 500 mg and vitamin D 1000 IU/day and oral pamidronate addition after 11 months of intravenous therapy (I .V. therapy) is also effective.[8]

Case Presentation

A 5-year-old boy presented to the clinic with progressive deformity in limbs (Figure 1, 2, 3) and pain during walking from the age of 2. He had also increased head circumference and a history of left humerus bone fracture at birth. The parents had no consanguinity.

Figure 1. Bowing of legs

In his physical examination, growth index was as below:

Weight: 16 kg (10%), Height: 109 cm (50%) and Head circumference: 53 cm

Laboratory tests were as below:

calcium: 10.6 mg/dl (NL:8.5–10.5)

phosphate: 5.3 mg/dl (NL:3.5–6.6)

alkaline phosphatase: 8211 U/L (NL:133-347)

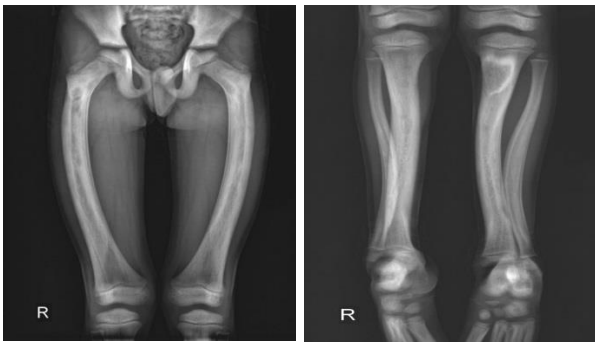

Figure 2. Bowing and thickening of the diaphysis and osteopenia

Radiographs showed large skull and thickened cranium with cotton wool appearance (Figure 4).

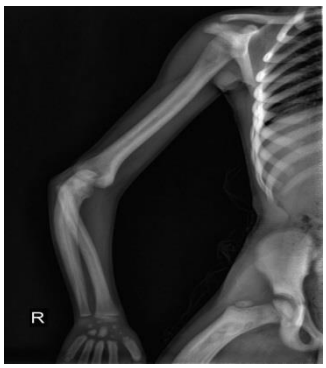

Figure 3. Deformity of ulna and radial bones is seen

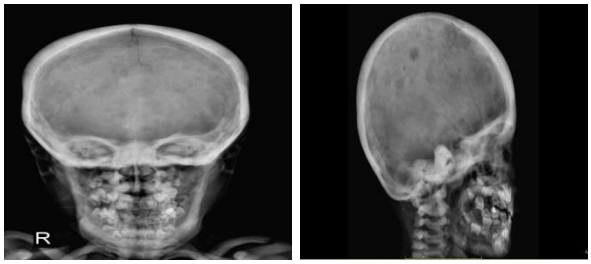

Figure 4. Skull is large and cranium is thickened with the cotton wool appearance

With the diagnosis of hyperphosphatasia, cyclical intravenous pamidronate (1 mg/kg/day during3 h, 3 consecutive days at 3-month intervals) started for him for 3.5 years. During treatment, limb deformities do not progress and he is good after 18 months follow up.

Discussion

We report a 5- year- old boy with progressive deformity of limbs and pain during walking from 2 years ago. With the diagnosis of hyperphosphatasia, he was treated with cyclical intravenous pamidronate (1mg/kg/day during 3 consecutive days at 3-month intervals for 3.5 years).

During treatment and 18 months follow up, limb deformity has shown no progress and bone pain has been resolved.

In similar studies, bisphonate (pamidronate) is selective treatment for improving growth rates and skeletal quality.[8]

Treatment with pamidronate is not a definitive treatment and due to the rarity of the disease, the authors of present article are interested in using the experiences of researchers.

Conclusion

Hereditary hyperphosphatasia is a rare autosomal recessive disorder (prevalence < 1 in 10 million). Its progressive skeletal deformities are associated with multiple fractures, which become apparent in the second or third year of life and result in dwarfing and sporadic cranial nerve involvement; loss of normal cortical outlines with the involvement of long bones from epiphysis to epiphysis. Serum calcium and phosphate levels are normal. The diagnosis establishes based on clinical, radiological, and histological features. Fractures, deformities, diffuse sclerosis on radiographs and high serum alkaline phosphatase is characteristic. As bisphosphonate can normalize bone turnover, improve growth rates, and skeletal quality, it is the treatment of choice for hyperphosphatasia.

Conflict of Interests

Authors have no conflict of interests.

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Case Report

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A Case Report of Familial Chylomicronemia Syndrome

Mahtab Ordooei[1,2], Fariba Binesh[3], Azam Ashrafi[2], Farzad Ferdosian[2,4,5*], Maryam Saeida-Ardekani[2]

[1] Hematology and Oncology Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[2] Mother and Newborn Health Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[3] Infectious Diseases Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[4] Children Growth Disorder Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[5] Department of Pediatrics, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

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Corresponding author:
Farzad Ferdosian

Email:
ferdosianfarzad@yahoo.com

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ABSTRACT

Background: Diagnosis of neonatal chylomicronemia, as a very rare condition, is very difficult and usually is diagnosed when acute pancreatitis sets in. Early diagnosis can prevent the complications such as acute pancreatitis and pancreatic necrosis which are associated with the condition.

Case Presentation: A 5.5 month- old female breastfed baby presented to us suffering from splenomegaly because of respiratory infection. Anemia and leukocytosis were seen in laboratory data. The result of bone marrow aspiration (BMA) performed to diagnosis was normal. Following the study, the patient had a high triglyceride, which improved with the treatment of symptoms and blood indices.

Conclusion: Our case reports a rare disorder that was initially admitted with suspicion of malignancy, organomegaly, anemia and leukocytosis. In the course of hospitalization, the diagnosis of malignancy was rejected after BMA, and chylomicronemia was diagnosed and the patient's leukocytosis and high uric acid were eliminated by treatment of the disease and the patient's symptoms were improved.

Introduction

Familial chylomicronemia syndrome (FCS) is a rare autosomal recessive disorder caused by mutations in lipoprotein lipase, apolipoprotein C-II deficiency or the presence of inhibitors to lipoprotein lipase, resulting in accumulation of chylomicrons in plasma and hypertriglyceridemia. There are several complications caused by elevated triglycerides in patients which acute pancreatitis is the most serious being episodes.[1-3] Due to its rarity and lack of specificity of signs and symptoms, the

recognition and correct diagnosis of the disease is challenging.[4] FCS is characterized by marked elevation of triglyceride and chylomicron levels, leading to lipemic plasma, recurrent attacks of acute pancreatitis, eruptive xanthoma, hepatosplenomegaly, lipemia retinalis.[2] Its prevalence is approximately 1 in 1 million for homozygotes.[5-7] Twenty- five percent of cases of familial chylomicronemia syndrome manifest during infancy; however, extremely rare cases manifest during the neonatal period. Pink- colored blood, milky white supernatant and falsely elevated pseudohyponatremia can be caused by severe hypertriglyceridemia. Lab studies usually show very high levels of triglycerides.[6] Treatment of patients with familial chylomicronemia requires severe dietary fat restriction to maintain fasting TG levels below 850 mg/dl to reduce the risk of pancreatitis.[8-10]

Case Presentation

The patient was a 5.5 month-old female baby of non-consanguineous parents admitted to Shahid Sadoughi hospital due to respiratory infection who was accidentally considered as suffering from splenomegaly in physical examination. Anemia and leukocytosis were found in laboratory tests. She did not have poor feeding, vomiting, restlessness, fever, any change in urination and defecation, diarrhea, constipation, and melena. The patient was the third child of a G3L3Ab0 mother created by a caesarian. Her parents had no relationship. There was no case history for the mother during pregnancy and she was also not on drugs during pregnancy. The patient's family history, medication-taking history and medical allergy were negative. The patient was breastfed and baby food was not started yet. She was pale, not jaundiced and didn't have a syndromic face. The fontanel was open, but not wide. The nasal bridge was not wide and the ears did not have a low set. The patient didn't have a cleft palate. The chest was not deformed in observation. There was no reduction in lung voice sound during auscultation. There were also no rales and wheezing, and sounds from two sides were normal and symmetrical in cardiac auscultation. There was no heart murmur and extra sound. No apparent lesion and scar was seen in stomach examination. No mass was touched. A splenomegaly about 6-5 cm under the edge of the ribs was touched. Hepatomegaly was not obvious.

In the patient laboratory data: The results of laboratory tests showed high uric acid, WBC, bilirubin and TG, and low hemoglobin (Table 1).

Table 1. laboratory data

Test	Preliminary result	Unit
BS	165	
urea	9	mg/dl
cr	0.4	mg/dl
Bili T	3.1	mg/dl
Bili D	1.1	mg/dl
ALT	30	U/L
AST	160	U/L
LDH	4546	U/L
Retic	1.8	
W.B.C	55700	3/ml^ X10
PLT	275000	
Hb	9.9	mg/dl
CRP	Weakly positive	mg/l
Coombs D & I	Negative	
Uric Acid	19	mg/dl
ESR	4	
CHOL	132	mg/dl
TG	1284	mg/dl
HDL	25	U/L
Na	135	mEq/L
K	3.3	mEq/L
Ferritin	112	ng/ml
Lipase	218	U/L
Amylase	26	U / ml

Peripheral blood smear: The patient's REC morphology

- Severe anisocytosis, hypochromia, dacryocytosis & schistocytosis
- 47NRBC /100WBC

The patients second PBC Sample

- RBC Morphology: Aniso + poikilo+ Hypo +Target+sphrocytosis +
- 3NRBC / 100WBC

Primarily the patient hospitalized at an

emergency department, considering the breast-fed signs the necessary tests requested, ultrasound was performed and peripheral blood smear (PBS) was requested. Numerous nucleated red blood cells (NRBCs) were observed in patient's PBS that patients' leukocytosis was justifiable based on. Because of the milky blood, cholesterol and triglyceride were checked. In the reported samples, patient had high TG and normal cholesterol. Considering endocrine consultative symptoms, medical history and tests related to the second blood sample which was taken before feeding, triglycerides and cholesterol check were requested again. The abnormality of chylomicronemia was recognized for the patient through repetition. Enzymatic and genetic studies were not performed for definitive diagnosis due to high cost. The patient was subjected to a special milk diet, MCT Oil and Omega3. During hospitalization, the patient had a drop in hemoglobin which leads to a PBS request again. Heart signs at consultation and Echo were normal and had an EF of 55%. During patient's monitoring for a week, the experiments had a downward trend.

- Chol: 159 mg/dl
- TG: 678 mg/dl
- WBC: 19900(Neu: 62% Lym: 20%)
- Hb: 10 (mcv: 75)
- PLT: 454000

A blood sample was also taken from patient's mother and father for checking triglycerides and cholesterol which both of them had a normal level.

Discussion

Hyperchilomicronemia leads to impaired laboratory test results. Because of thinning the membrane of red blood cells, hemolysis occurs more rapidly that leads to hemolytic anemia. In the mentioned case, there was high leukocytosis and uric acid and an increase in LDH according to the signs and laboratory results. At first, the patient was approached with suspected malignancy. Malignancy was rejected due to the normal bone marrow result after necessary examinations. The patient was treated for hyperchilomicronemia according to the results of tests during the hospitalization and diagnosis. After the treatment, the results of tests and hemolytic anemia were improving during the follow-up care. According to the results of the treatment, it can be concluded that an increase in blood triglyceride leads to disorders such as hemolytic anemia and leukocytosis and an increase in uric acid in laboratory studies. A similar result is reviewed in another report case.[11]

Conclusion

High triglyceride can cause symptoms similar to hemolytic anemia and leukocytosis. Ruling out the malignancy, treatment can be started and normalization of blood indices has prospected in the follow-up.

Conflict of Interests

Authors have no conflict of interests.

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