**Review Article**

**Zika Virus Induced Microcephaly in Pregnancy: Is There a Specific Prenatal Biomarker Screen Available? Review and Commentary**

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**Abstract:**

The recent Zika virus outbreak, which began in Brazil in 2015, has been linked to microcephaly in fetuses and newborns. The virus has spread rapidly through South, Central, and even North America; hence, it has been declared a public health emergency of international concern. Presently, a specific prenatal biomarker screen for microcephaly is not in place because the cranial defect usually appears among a cluster of neural anatomic developmental malformations of the cerebrum. Although the present prenatal screen for Down syndrome (DS) in the second trimester could be utilized as a substitute triple marker biomarker assay, the risk calculations are not specific for microcephaly. Moreover, prenatal DS testing sometimes produces a negative screen and microcephaly has previously been misdiagnosed as an anencephaly. Therefore, a modified triple test for microcephaly has been suggested for the development of a more specific prenatal screening test in the second trimester of pregnancy.

**Keywords:** Alpha-Fetoprotein; Estradiol; Estriol; Human Chorionic Gonadotrophin; Microcephaly; Triple Test; Zika Virus

**Abbreviations:**

- AFP: Alpha-Fetoprotein
- ASPM: Abnormal Spindle Microcephaly-Associated Protein
- Cas-9: Endonuclease Complex of Two RNA Molecules
- CdK5 RAP2: CDC2 Kinase Complexed t CdK5
- CRISPR: Clustered Regularly-Interspaced Short Palindromic Repeats
- DS: Down Syndrome
- EMC: Membrane Protein Complex
- ER: Endoplasmic Reticulum
- hCG: Human Chorionic Gonadotrophin
- IFITM3: Interferon-Induced Transmembrane Protein-3
- IUGR: Intrauterine Growth Retardation
- MCP: Microcephaly
- MCPH1: microcephalin
- MOM: Multiple of Median
- NTD: Neural Tube Defect
- PAPP-A: Pregnancy Associated Plasma Protein-A
- RNAi: RNA Interference Molecule that Inhibits Gene Expression
- TXL: Tyrosine Kinase Receptor
- WDR62: WD Repeat Containing Protein-62
- ZIKV: Zika Virus

**Introduction:**

The Zika virus (ZIKV) Flavivirus from the family Flaviviridae is a worldwide emerging infectious agent transmitted by the mosquito *Aedes aegypti* [1]. The RNA arbovirus was first isolated in 1947 from the blood of a Rhesus macaque monkey in the Zika forest of Uganda, Africa [2]. In adults, the symptoms of ZIKV infection are similar to dengue fever exhibiting fever, headache, arthralgia, myalgia, and maculopapular rash [3]. ZIKV has also been linked to the adult autoimmune neurological Guillain-Barré syndrome. There are currently no vaccine and antiviral treatments available for ZIKV infections. Thus, ZIKV infection is rapidly becoming a public health emergency of international concern. Recent outbreaks in North, Central, and South Americas, the Caribbean, and French Polynesia have shown an increased incidence of microcephaly (MCP) during pregnancy [4]. MCP is defined as a fetal head circumference more than three standard deviations below the mean [5]. The target tissue of the ZIKV in pregnancy is the brain, especially the cerebral cortex. MCP can have a prenatal or postnatal onset and often occurs in association with other brain malformations and/or disruptions in any one of the three trimesters. The incidence for non-viral induced or congenital MCP is reported as 1 in 6,000 to 10,000 births [6]. Most if not all cases of MCP result in moderate to severe mental retardation of the patient.
MCP in early pregnancy or at term displays cranial growth retardation together with cerebral cortex cell necrosis and atrophy resulting in a “dwarfed brain” [7]. Such fetal brain dysfunctions can contribute to hydrocephalus, holoprosencephaly, ventriculomegaly, cerebral calcifications, excessive extra-cerebral fluid formation, ocular abnormalities, and cerebral agenesis [8]. Postnatally, the neonates display microcephaly, triangular face, hypertelorism, epicanthic folds, low-set ears, and micrognathia [9]. Individuals with this disorder have a smaller head size than normal, intellectual disability, poor motor function, abnormal facial features, seizures, gonadal failure, and short stature (dwarfism). MCP can result from: a) virus infections; b) aneuploidies (chromosome abnormalities); c) gene mutations; d) cell cycle mitotic dysfunction, and e) chromosome instability syndromes (DNA repair disorders). Many brain lesions produced by viruses such as Zika resemble those of congenital cytomegalovirus, toxoplasmosis, rubella, choriomeningitis, and dengue fever [3]. The specific targets of ZIKV in MCP are cells comprising the cerebrum and developing telencephalon, which result in the disruption of neurogenesis in the cerebral cortex. Thus, ZIKV infection is associated more with disruption in brain development rather than destruction of the brain.

Objective: Presently, there exists no prenatal screening test platform and algorithm specifically designed to detect MCP because it is usually reported as a collateral birth defect among a cluster of neuro-anatomical malformations. Other than substituting a Down syndrome (DS) triple or quad screening test for ZIKV infection in pregnancy, present day biomarker protocols lack a specific prenatal screening test for MCP. However, MCP is not DS and the risk factors calculated for DS do not specifically apply to the risk of having a MCP-associated pregnancy. The lack of a prenatal screening test for MCP in early pregnancy following a ZIKV infection presents a pressing unmet public health need both in the Western Hemisphere and worldwide [10,11]. Hence, the objective of this commentary is to forward a call of alert for public health awareness and preparedness concerning the need for an appropriate prenatal screen for MCP in pregnancy. In so doing, this commentary will present suggestions for developing a prenatal biomarker profile specifically designed for MCP screening in second trimester pregnancies.

Characteristics and Factors Contributing to Microcephaly Formation: Interestingly, MCP has been mistakenly diagnosed as an anencephaly which constitutes a different cranial neural tube defect (see later discussion). However, MCP is not a neural tube defect, the latter of which results from non-closure of the neural tube during early embryonic development. The neural tube defects include: a) anencephaly; b) encephalocele; c) meningomyelecele; and d) spina bifida. All open neural tube defects display highly elevated alpha-fetoprotein (AFP) levels due to leakage of AFP into the amniotic fluid compartment and its passage into the maternal circulation [12]. In contrast, MCP is a cerebral cortex abnormality unrelated to neural tube formation and unrelated to AFP leakage into the amniotic fluid and maternal circulation.

The MCP defect initially forms just prior to the 12th week of gestation at which time cell damage has already occurred in the cerebral cortex of the fetal brain as a result of cortical cell necrosis [13]. The cell death is followed by a decrease in intracranial pressure and collapse of the roof of the fetal skull [14,15]. As a result of cranial collapse, a loosening, detachment and overlapping of the cranial suture network then ensues. To date, three possible causes of MCP malformations have been proposed; namely, 1) vascular disruption; 2) intrauterine cerebral infarction; and 3) viral infections as presently discussed [16].

The cellular factors contributing to MCP have not been fully elucidated, but several detrimental biochemical events underlying fetal brain size regulation have been reported. A critical event in cerebral cortex cell dysfunction appears to be cell cycle mitotic disruptions and perturbations. Impaired mitosis is expressed cytogenetically as an increased frequency of premature chromatid separation [17]. The mitotic phase disruptions can include the following: a) untimely entry into the mitotic phase of the cycle; b) inappropriate cell cycle exit; c) premature chromosome condensation in early G2 phase; d) cell accumulation and stagnation in the mitotic prophase stage of cell division; e) disruptions in spindle fiber formation; f) dysfunctional centrosomal proteins; g) mutated genes encoding a kinetochore protein responsible for attachment to the mitotic spindle, h) activation of the spindle check point pathway; i) improper chromosome aggregation; and j) mutation and disruption of the cytoskeletal beta-tubulin gene [18-23]. Overall, MCP appears to be a result of several major factors. These factors can be summarized as cell cycle dysregulation of mitosis, mitotic arrest due to disruption of centrosomal and spindle fiber formation, and altered DNA repair pathways [24].
Although the placenta attempts to mount a defensive response against the virus, it is a failed endeavor and ZIKV is able to evade immune surveillance [8]. As a result of virus transfection, calcifications occur in the placenta and later in the cerebral cortex. In the cerebrum affected by microcephaly, enlargement of the ventricles takes place (ventricomegaly) accompanied by the occurrence of cell agenesis in the cerebral cortex.

Microcephaly often occurs concurrent with chromosomal aneuploidies which are a major cause of brain size reduction due to a decrease in proliferating neural stem cells. MCP is known to occur concurrent with aneuploidies such as: a) a terminal deletion on chromosome 7q [30]; b) trisomy 6p [31]; c) mosaic distal chromosome 5p deletion [9]; d) interstitial deletion of chromosome 2 (p23, p25) [32], and the long arm of chromosome-13q [33]. It is of special interest that such aneuploidies display highly elevated levels of human chorionic gonadotrophin (hCG). In one clinical case, a pregnant woman whose fetus had a chromosome 7q deletion exhibited an hCG level of MOM = 8.7 [30]. hCG is a trophic hormone produced by the placenta which is known to have a reciprocal relationship with the placental aromatase-derived estradiol (E2). That is, high E2 concentrations can act to suppress hCG to low levels, while low levels of E2 are concurrent with the presence of high hCG concentrations [34]. Steroidal E2 levels normally rise during pregnancy and contribute to the rapid decline of hCG by week 20 of gestation. E2 suppresses the hCG levels at the time of gonad differentiation of the cortical and medullary sex cords of the primordial gonads. In the case of MCP, gonadal development is disrupted (gonadal failure; hypogonadism), E2 levels remain low, and hCG levels are elevated [35,36]. In theory, maternal serum screening levels in women bearing microcephalic fetuses should display a steroid biomarker profile of low E2 accompanied by highly elevated hCG levels.

It is of further interest that E2 can suppress the expression of genes regulating brain size in both humans and primates and influence apoptotic neurodegeneration [37,38]. E2 (in animal models) has also been shown to induce MCP by modulating four genes involved in the regulation of brain size and volume, namely; MCPH1, ASPM, Cdk5 RAP2, and WCR62 (see below). As a result, lowered E2 is present in the brain concurrent with a reduced number of E2 receptors both of which could contribute to cerebral cortex size reduction. Thus, E2 levels should be reduced in pregnancies with a microcephalicfetal brain.
Could MCP in Pregnancy Be Detected by Present-Day Prenatal Triple or Quad Screening for Down Syndrome?

Present-day prenatal triple screen testing for second trimester Down syndrome consists of: 1) reduced AFP levels; 2) elevated hCG levels; and 3) low levels of unconjugated estriol (E3). Could MCP be detected by the present triple test? The DYRK1A tyrosine kinase gene, located on chromosome 21q22.13 lies within a Down syndrome critical region [47]. Some Down syndrome fetuses with this mutation exhibit MCP as a primary or acquired defect. Nonetheless, DS screening is not specific for MCP and this brain defect might only appear among other associated abnormalities in DS fetuses. Moreover, a DS screen in a pregnant woman with an MCP might not result in a significant risk for Down syndrome using a triple test, depending on the analyte concentrations. It is of interest that estriol (E3) is not involved in MCP malformations and would constitute a non-interacting neutral component. Furthermore, dimeric inhibin-A is not associated with the development of MCP formation; thus a quad screen would not be necessary. A subsequent follow-up with ultrasound and associated imaging procedures might be capable of detecting a MCP brain lesion. Second trimester ultrasound should be able to detect MCP but can be limited since false positive and false negative diagnoses still occur [6]. Nonetheless, as stated above, the risk calculations for Down syndrome, using triple test analytes would not be specific for MCP.

Regarding a first trimester screen for MCP, the use of the present Down syndrome screen utilizing high hCG, low PAPP-A, and nuchal translucency might suffice. As previously shown, hCG is highly elevated in the presence of MCP and PAPP-A has recently been shown to be low in two reported cases of MCP [31,48]. This constitutes the classical two analyte profile for DS first trimester screening. Nuchal translucency further contributes to obtaining a positive screen result for DS in the 11th-14th week of gestation, but its usefulness for MCP has yet to be reported and confirmed.

What Could Constitute a More Specific Prenatal Screening Panel for MCP?

In view of the above discourse, a new triple test profile for prenatal screening for MCP can presently be proposed. A literature survey and study of MCP leads one to propose that a biomarker profile might consist of the following components:

MCPH1, termed microcephalin, is a gene expressed during fetal brain development, which when mutated, causes MCP [39]. ASPM, called abnormal spindle microcephaly-associated protein; when mutated, the defective form of ASPM is associated with an autosomal recessive form of primary microcephaly [40]. CdK5-RAP2 is a neuronal CDC2-like kinase in complex with the catalytic subunit of CdK5; it is involved in neuronal differentiation of cortical cell surface area and total brain volume [41]. WDR62 is a gene named WD repeat-containing protein-62 whose mutations cause severe cerebral cortex malformations including MCP; cortical thickening, and hypoplasia of the corpus callosum [42].

Microcephaly and Alpha-fetoprotein: In general, low AFP levels in a microcephaly-associated pregnancy was reported by Villoe et al. in a severe case of MCP at 21 weeks [15]. AFP levels were found to be very low in both intrauterine growth retardation (IUGR) and in brain growth restriction. In a further case report, a deletion of chromosome-2 associated with MCP exhibited an AFP level which was undetectable at 18 weeks [32]. In an Angelman’s syndrome case with MCP at 16 weeks, very low AFP levels (MOM = 0.23) were reported in the presence of an MCP defect together with a chromosome 15q deletion [43]. In another case of brain disruption with MCP, very low AFP levels were noted at 21 weeks gestation [13]. Salihu et al. studied a case of trisomy-18 in a fetus with MCP which also demonstrated low AFP levels [44]. In another reported instance, an ultrasound result at 26 weeks was mistakenly diagnosed as a MCP in an anencephalic fetus even though the AFP levels were low and not elevated [45]. Lastly, a low AFP value was described in a case of MCP at 16 weeks in a MCP-affected fetus with Galloway Mowat syndrome [46]. In contrast to the above AFP levels, Chen et al. reported a case of MCP in a patient with a deletion of chromosome 7q displaying normal AFP levels (MOM = 1.02) accompanied by an elevated hCG MOM of 8.6 [30].
1) maternal serum AFP (MS) at undetectable, low, or low/normal levels; 2) extremely elevated levels of MS hCG; and 3) reduced levels of MS estradiol. Pregnancy-derived estradiol concentrations are a product of the placenta involved in a maternal/placental/fetal axis and frequently reflects the reciprocal of hCG levels as shown above. MCP results from a collapsed roof of the fetal skull positioned above the cerebral cortex followed by cortical cell necrosis and atrophy; these events result in decreased cortical cell populations. Reduced cortex cell numbers could also signify less uptake of E2 in brain cells. Although AFP is not synthesized and secreted by brain cells, AFP is readily taken up by the brain and is found abundantly in such fetal cells [49]. AFP is known to control female fertility and the gonadotropic-releasing hormone pathway through an anti-estrogenic (E2) action [50]. Reduced levels of AFP in the blood vessels and cortical cells of the MCP developing brain might contribute to the observed low levels of AFP found in microcephalic-associated pregnancies.

Conclusion: It is evident from the above commentary that Zika virus-induced MCP during pregnancy and the lack of a specific screening assay, represents a major unmet public health need in the world-wide medical community. Although the present day prenatal triple screen for Down syndrome could be utilized as a stopgap substitute screen for MCP in first and second trimesters, the development of a more specific triple test utilizing very low AFP, very high hCG, and reduced E2 levels is presently suggested.

References:


