ABSTRACT: A paper in the current issue of *Fluoride* by Changalvala et al. reports a new finding of raised serum fluoride levels in pregnant women with pre-eclampsia. The authors found that pregnant women, at 20 or more weeks of gestation, with pre-eclampsia (n=150) in Karnataka, India, had significantly higher levels of serum fluoride (p<0.05) than a control group of pregnant women without pre-eclampsia (n=150) (1.8±0.66 and 0.18±0.31 mg/L, respectively). The question follows as to whether the association between a raised serum fluoride and pre-eclampsia is causal. The current knowledge on pre-eclampsia indicates that it is a complex multisystem disease. Central to the pathogenesis are abnormal placentation with uteroplacental ischaemia early in the first trimester and a hypertensive maternal syndrome with multi-organ failure, later in the second and third trimesters, with the release into the maternal circulation from the ischaemic placenta of soluble toxic and antiangiogenic factors, such as soluble fms-like tyrosine kinase (sFLT1) and soluble endoglin (sENG), which result in inflammation, endothelial cell dysfunction, and maternal systemic disease. The placental ischaemia may lead to small for gestational age babies. Various theories have been proposed to explain the condition. To help establish whether or not the relationship between fluoride exposure and pre-eclampsia might be one of causation rather than association, two of the nine Bradford Hill criteria establishing a causal relationship were considered. When coherence was examined with respect to low birth weight, the generally known facts of the natural history and biology of fluorosis, and pre-eclampsia, no serious conflict was found. The criterion of plausibility was examined by considering whether or not there were known effects of fluoride that might affect the pathophysiology as currently understood. The result was that it was considered plausible that fluoride might be able to affect the development of the disease. At present, on this basis, it is more likely than not that fluoride is a risk factor for the development of pre-eclampsia. However, the pathophysiology of pre-eclampsia is complex and will become clearer over time as more research is done.

Keywords: Coherence; Pathophysiology; Plausibility; Pre-eclampsia, Serum fluoride.

A paper in the current issue of *Fluoride* by Changalvala et al. reports a new finding of raised serum fluoride levels in pregnant women with pre-eclampsia. The authors found that pregnant women, at 20 or more weeks of gestation, with pre-eclampsia (n=150) in Karnataka, India, had significantly higher levels of serum fluoride (p<0.05) than a control group of pregnant women without pre-eclampsia (n=150) (1.8±0.66 and 0.18±0.31 mg/L, respectively). The question follows as to whether the association between a raised serum fluoride and pre-eclampsia is causal.

Bradford Hill observed, in 1945, that proof that A and B are associated is not proof that a change in A is directly responsible for a change in B or vice versa. There might be some common factor C which was responsible for their associated movements. As an example, he commented that, although the death rate from cancer in England and Wales had been rising at the same time as the sale of bananas, no one had deduced, as far as he knew, from this relationship in time that there was a relationship of cause and effect. He suggested that there were nine aspects of an association between two variables that should be especially considered before deciding that the most likely interpretation of it was causation: (i) strength, (ii) consistency, (iii) specificity, (iv) temporality, (v) biological gradient, (vi) plausibility, (vii) coherence, (viii) experiment, and (ix) analogy.

*Coherence:* In considering the aspect of the Bradford Hill factor of coherence, or whether the data on fluoride and pre-eclampsia seriously conflicts with the generally
known facts of the natural history and biology of fluorosis and pre-eclampsia, it is noted that pre-eclampsia causes intrauterine growth restriction and the complications for the foetus include low birth weight. Several studies from India have found a relationship between a high fluoride intake and low birth weight. Susheela and Susheela et al. found, in 2010, that the incidence of low birth weight (<2.5 kg) was significantly reduced (p<0.0001) to a value of less than half in pregnant women (n=90) with severe anaemia (Hb=5.0–9.0 g/dL) and high urinary fluoride (>1 mg/L) who received dietary editing and counselling, starting in the first and second trimesters, for reducing fluoride intake and ensuring an adequate intake of essential nutrients (viz., calcium, vitamins, and antioxidants from dairy products, fruits, and vegetables) compared to a control group (n=115) (edited and counselled group: 1st trimester start=20%, 2nd trimester start=23%; control group: 1st trimester start=51%, 2nd trimester start=53%). The nutritional intervention also resulted in a nonsignificant reduction in the urinary fluoride level (p>0.01) (edited and counselled group: 1st trimester start=67%, 2nd trimester start=53%; control group: 1st trimester start=49%, 2nd trimester start=37%), a significant increase in Hb (p<0.001) (edited and counselled group: 1st trimester start=73%, 2nd trimester start=83%; control group: 1st trimester start=59%, 2nd trimester start=54%), a reduction in pre-term delivery (<34 weeks gestation) (edited and counselled group=2%; control group=8%), and an increase in full term deliveries (edited and counselled group=68%, control group=50%). Similarly, Susheela and Kumari found, in 2020, that the incidence of low birth weight (<2.5 kg) was 17% in pregnant women (n=234) with anaemia (Hb<12 g/dL) and high urinary fluoride (>1 mg/L) who received dietary editing and counselling, compared to the incidence of low birth weight in a control group (n=247) of 41%. Susheela et al. considered that maternal and child under-nutrition and anaemia is not necessarily due to insufficient food intake but because of the derangement of nutrient absorption due to damage caused to the gastrointestinal (GI) mucosa by ingestion of an undesirable chemical substance, viz., fluoride through food, water, and other sources. They considered that their approach to anaemia of pregnancy, by reducing fluoride intake and ensuring an adequate intake of essential nutrients, could reduce the percentage of low birth weight babies and consequently the burden of disabled and mentally challenged children.

In a study of 108 apparently healthy pregnant women, aged 17–36 yr, in the Nalgonda District, Andhra Pradesh, India, Sastry et al. found, in 2011, that when the maternal serum fluoride was greater than 1 ppm the relative risk of low birth weight (<2.5 kg) was increased by ×10.58 (p<0.0001) and the risk of preterm delivery was increased by ×8.65. When the umbilical cord serum fluoride was greater than 0.22 ppm the risk of low birth weight (<2.5 kg) was increased by ×2.76 (p<0.0001) and the risk of preterm delivery was increased by ×4.6 (p<0.001). Sastry et al. did not measure the maternal Hb but considered that the study of Susheela et al. was supportive of their findings and gave a plausible hypothesis to explain the relationship: the increase in the risk of birth weight and preterm delivery was the result of fluoride-induced anaemia.

In a case-control study by Diouf et al. in 2012, 108 mothers who gave birth to low birth weight newborns (<2.5 kg) in the Diourbel region of Senegal, an endemic dental fluorosis region with water concentrations of for drill water and well water of 4.7 and
0.009 mg/L, respectively, were compared to 216 mothers whose newborns weighed ≥2.5 kg. According to Table 2 in the paper, more of the cases consumed drilled water with a fluoride level of 4.7 mg/L (62%) than the controls (43.5%) although the difference was not significant (p=0.07). It is noted that there appears to be an error in the abstract which refers to 62% of the cases and 43.5% of the controls consuming well water, whereas the figures in Table 2 for well water consumption are 27.8% and 42.6%, respectively. A score of 4 on Dean’s index for dental fluorosis in the mothers was found for 25.9% of the cases and 6.9% of the controls (p=0.029). The authors found, using univariate analysis, that low birth weight was significantly associated with the presence of consanguinity (cases=48.1%, controls=28.7%, p=0.0005), anaemia (cases=24.1%, controls=9.3%, p=0.0003), hypertension (cases=23.1%, controls=11.6%, p=0.007), and pre-eclampsia (cases=23.1%, controls=11.6%, p=0.007). Using multivariate analysis and adjusting for the other variables, the relative risks (RRs) and 95% confidence intervals (CIs) for having a low birth weight newborn were: (i) type of water ingested: mineral water—RR=1, 95% CI=1; well water—RR=0.88, 95% CI=0.5–2.51; and drill water—RR=1.99, 95% CI=1.3–3.67, p=0.04; (ii) Dean’s index: 0—RR=1; 1—RR=0.31, 95% CI=0.11–0.91; 2—RR=0.66, 95% CI=0.23–1.96; 3—RR=2.77, 95% CI=0.85–9.02; 4—RR=3.2, 95% CI=1.1–9.43, p=0.001; (iii) parity (number of pregnancies reaching a viable gestational age): unit—RR=1.2, 95% CI=1.03–1.39, p=0.017; (iv) consanguinity: no—RR=1, yes—RR=2.04, 95% CI=1.18–3.25, p=0.011; (v) anaemia: no—RR=1, yes—RR=3.7, 95% CI=1.78–7.72, p<0.01, (vi) hypertension: no—RR=1, yes—RR=2.75, 95% CI=1.35–5.61, p=0.005. This study supports anaemia being an important mechanism for fluoride-induced low birth weight (RR=3.7) but suggests that there is still some risk independent of the presence of anaemia (RR=1.99) for consuming drinking water with a high fluoride content (4.7 mg/L).

The data from these studies on fluoride and low birth weight are not in serious conflict with the generally known facts of the natural history and biology of fluorosis and pre-eclampsia and indicate that a degree of coherence is present.

Plausibility: Bradford Hill suggested that it would be helpful for establishing a causal relationship if the suspected causation was biologically plausible. To examine this it is necessary to consider the pathophysiology of pre-eclampsia.

Pre-eclampsia affects 5% to 7% of all pregnant women and is responsible for over 70,000 maternal deaths and 500,000 fetal deaths worldwide every year. Pre-eclampsia was classically defined by the American College of Obstetrics and Gynecology (ACOG) as the presence of hypertension and proteinuria occurring after 20 weeks of gestation in a previously normotensive patient. However, because some women developed systemic manifestations of pre-eclampsia, such as thrombocytopenia or elevated liver enzymes, before proteinuria was detectable, the condition is now understood to be a heterogeneous hypertensive disorder of pregnancy and the definition was revised in 2013 in the ACOG practice guidelines to include the presence of severe features with or without proteinuria and to exclude the degree of proteinuria as a criterion for a severe feature. The ACOG clinical definition of pre-eclampsia now involves, as listed below, either (i) the presence of increased blood pressure and proteinuria, or (ii) the presence of increased blood pressure and severe features:
Fluoride exposure was not mentioned as a risk factor.9 Other rarer risk factors included genetic susceptibility in the mother or father, family history of pre-eclampsia, and a trisomy 13 foetus.

Rana et al. considered that pre-eclampsia was a placental disease with two stages: (i) stage 1: abnormal placentation with uteroplacental ischaemia early in the first trimester and (ii) stage 2: a maternal syndrome later in the second and third trimesters with an excess of antiangiogenic factors.9 The theories proposed for the abnormal placentation include oxidative stress, abnormal natural killer cells (NKs) at the maternal-fetal interface, and genetic and environmental factors. Animal models suggest that the uteroplacental ischemia drives the hypertensive, multi-organ failure response observed in the maternal pre-eclamptic syndrome in stage 2. The diseased placenta is considered to release soluble toxic factors in the maternal circulation that result in inflammation, endothelial dysfunction, and maternal systemic disease. The placental ischaemia may lead to small for gestational age babies.

**PATHOGENESIS OF STAGE 1 OF PRE-ECLAMPSIA:**

(i) Impaired cytotrophoblast transformation from the proliferative epithelial subtype to the invasive epithelial subtype: In describing the pathogenesis of stage 1 of pre-eclampsia, involving abnormal placentation, trophoblast invasion, and the
maternal-fetal interface, Rana et al. noted that during normal placental implantation, cytotrophoblasts migrated into the maternal uterine spiral arteries to form vascular sinuses at the fetal-maternal interface which provided nutrition to the fetus. In normal pregnancy, this invasion progressed deeply into the spiral artery to the level of the myometrium and led to extensive remodeling of the maternal spiral arterioles into high capacitance, high flow vessels. In the placentas associated with pre-eclampsia, the cytotrophoblasts failed to transform from the proliferative epithelial subtype to the invasive endothelial subtype leading to an incomplete remodeling of the spiral artery with narrow maternal vessels and relative placental ischemia.

(ii) Atherosclerosis of uterine spiral arteries: The narrow spiral arteries were prone to atherosclerosis, with lipid-laden macrophages in the lumen, fibrinoid necrosis of the arterial wall, and a mononuclear perivascular infiltrate, leading to a further compromise in placental flow. Women with pre-eclampsia have a significant impairment of diastolic flow in the spiral arteries with a characteristic notch in the waveform on Doppler ultrasound examination that antedates the clinical signs and symptoms of pre-eclampsia. These findings suggest that an abnormality in the trophoblasts themselves may result in shallow placentation and inadequate transformation of the spiral arteries, leading to placental ischemia and the maternal syndrome of pre-eclampsia.

(iii) Atherosclerosis of uterine radial arteries: Atherosclerotic changes in the maternal radial arteries that supply the decidua—as opposed to the spiral arteries—are also observed in pre-eclampsia. Decidual vasculopathy is a lesion common to disorders of placental insufficiency, including intrauterine growth restriction and pre-eclampsia, and combines (i) acute atherosclerotic lesions with (ii) medial hypertrophy and perivascular lymphocytes. Within pre-eclampsia phenotypes, the presence of decidual vasculopathy is associated with worse clinical outcome, higher diastolic BP, worse renal function, and perinatal fetal death. Histologically, normal third-trimester decidual vessels are characterized by flat endothelium and a loss of medial smooth muscle, while pre-eclamptic decidua show signs of loose, edematous endothelium, hypertrophy of the vessel media, and a loss of smooth muscle modifications as seen in atherosclerosis.

(iv) Impaired stromal transformation of uterine endometrium to prepare for implantation: In addition to uteroplacental insufficiency, epidemiological studies suggest that poor uterine decidualization—the stromal transformation of uterine endometrium to prepare for implantation—may affect the development of pre-eclampsia. (i) global transcriptional profiling of chorionic villus samples points to insufficient or defective decidualization in pregnancies that were later complicated by severe pre-eclampsia; (ii) endometrial stromal cells from nonpregnant donors with a history of severe pre-eclampsia fail to decidualize in vitro and are transcriptionally inert, suggesting baseline genetic abnormalities or genetic modifications; and (iii) global transcriptional profiling of decidual tissue from the women with pre-eclampsia also revealed defects in gene expression. These cells failed to redecidualize in culture, and their conditioned medium failed to support cytotrophoblast invasion, suggesting that decidual cells may be an important contributor to downregulated cytotrophoblast invasion in pre-eclampsia. Defective placentation might be the result of combinations of factors that affect both trophoblast and decidua.
(v) Overexpression of hypoxia-inducible transcription factors HIF-1α: and HIF-2α: Upregulation of hypoxia-inducible transcription factors (TFs) and hypoxia-related gene signatures in the placenta suggest that hypoxia is central to the pathogenesis of pre-eclampsia. In the early phases of implantation, the gestational sac exists in a low oxygen tension environment, favoring trophoblast proliferation. Before the invasion, the proliferating trophoblasts anchor the blastocyst to maternal tissues and plug the tips of the spiral arteries within the decidua. Eventually these trophoblastic-spiral artery plugs collapse, forming an intervillous space. The newly formed sinuses allow for the arrival of maternal blood, increasing oxygen tension, generating oxidative stress, and promoting trophoblast differentiation from a proliferative to an invasive phenotype that will invade and remodel the spiral arteries. HIF (hypoxia-inducible factors)-1α and -2α, markers of cellular oxygen deprivation, are expressed at high levels in proliferative trophoblasts and in the placentas of women with pre-eclampsia. Overexpression of HIF-1α in pregnant mice is associated with hypertension, proteinuria, and fetal growth restriction in mice, and may result in failure of trophoblastic differentiation from the proliferative to the invasive phenotype. Furthermore, inhibition of HIF-1α by 2-methoxyestradiol, a metabolite of estradiol that destabilizes HIF-1α, suppresses the production of sFLT1 (soluble fms-like tyrosine kinase 1), a potent antiangiogenic factor known to contribute to the maternal syndrome.

(vi) Oxidative stress with an imbalance of reactive oxygen species (ROS)-generating enzymes and antioxidants from mitochondrial stress and endoplasmic reticulum stress: While low oxygen tension followed by maternal blood flow oxygenation results in normal placentation, intermittent hypoxia and reoxygenation caused by poor spiral artery invasion may cause oxidative stress. At the molecular level, pre-eclamptic placentas show an imbalance of reactive oxygen species (ROS)-generating enzymes and antioxidants. Oxidative stress may also promote the transcription of antiangiogenic factors such as sFLT1. In humans, placental antioxidant mechanisms are impaired in patients with pre-eclampsia, as shown by their decreased expression of superoxide dismutase and glutathione peroxidase compared with women with normal pregnancies.

ROS may derive from mitochondrial stress. The activity of the mitochondrial electron transport chain (ETC) enzyme cytochrome C oxidase is decreased in the syncytiotrophoblast cells of pre-eclamptic placentas, which correlated with increased placental sFLT1 expression. Hydrogen sulfide donors inhibit HIF-1α, and pretreatment with AP39, a mitochondrial-targeting hydrogen sulfide donor, can decrease sFLT1 expression in human syncytiotrophoblasts and increase cytochrome C oxidase activity in a dose-dependent fashion in normal and pre-eclamptic placentas, thus preventing the release of ROS and leading to the subsequent stabilization of HIF-1α.

Another possible source of oxidative stress is endoplasmic reticulum stress caused by ischemia-reperfusion injury. Endoplasmic reticulum stress has been observed in the decidua and placentas of patients with fetal growth restriction and pre-eclampsia and triggers decidual cell and cytotrophoblast apoptosis through the activation of the unfolded protein response (UPR). PERK (PKR-like endoplasmic reticulum kinase), a transmembrane kinase that decreases the translational burden of the endoplasmic
Fluoride as a risk factor in pre-eclampsia

Spittle

PATHOGENESIS OF STAGE 2 OF PRE-ECLAMPSIA:

(i) Increased expression of the antiangiogenic protein sFLT1: In describing the pathogenesis of stage 2 of pre-eclampsia, involving the pathogenesis of the maternal syndrome, Rana et al. considered an imbalance in circulating angiogenic factors and noted that elevated levels of the antiangiogenic protein sFLT1 have been identified in placentas collected from women with a clinical diagnosis of pre-eclampsia. sFLT1 is a soluble protein that exerts antiangiogenic effects by binding to and inhibiting the biological activity of proangiogenic proteins VEGF and PlGF. VEGF is important for the maintenance of endothelial cell function, especially in fenestrated endothelium, which is found in the brain, liver, and glomeruli, the primary organs affected by pre-eclampsia. A member of the VEGF family, PlGF is important in angiogenesis and selectively binds to VEGFR1/sFLT1 not VEGFR2. Several findings implicated sFLT1 in the pathogenesis of pre-eclampsia: (i) sFLT1 protein levels were high in maternal plasma or serum; (ii) sFLT1 mRNA expression was high in pre-eclamptic placentas; and (iii) injecting exogenous sFLT1 into rodents led to hypertension, proteinuria, glomerular endotheliosis (a hallmark of pre-eclampsia seen in renal biopsy), as well as several other pre-eclamptic features; (iv) treatment of cancer patients with anti-VEGF drugs results in hypertension and proteinuria; (v)
depletion of sFLT1 in pre-eclamptic plasma using antibodies reverses the antiangiogenic phenotype in cell culture studies; (vi) lowering sFLT1 or antagonizing sFLT1 in animal models of pre-eclampsia improves clinical symptoms; (vii) spontaneous resolution of clinical signs and symptoms of pre-eclampsia, when sFLT1 levels are lowered by 50% or more by treatment of the underlying placental conditions such as fetal hydrops or removal of diseased placenta in multiple pregnancies; and (viii) in addition to the elevated sFLT1 levels, the circulating levels of free PlGF were reduced in women with pre-eclampsia, suggesting an imbalance of antiangiogenic and proangiogenic proteins.9

(ii) Increased expression of the antiangiogenic protein soluble endoglin (sENG): Another antiangiogenic protein that has also been extensively studied in pre-eclampsia is soluble endoglin (sENG), an endogenous TGF-β1 (transforming growth factor β1) inhibitor.9 sENG is elevated in the sera of pre-eclamptic women 2 months before the onset of clinical signs of pre-eclampsia, correlates with disease severity, and falls after delivery.9 In pregnant rats, it appears to potentiate the vascular effects of sFLT1 to induce a severe pre-eclampsia-like state, including the development of thrombocytopenia and fetal growth restriction, and, in combination with sFLT1, appears to induce cerebral edema resembling the reversible posterior leukoencephalopathy seen in patients with eclampsia.9

(iii) Reduced expression of interleukin 10: In considering inflammatory cytokines and immune cell alterations, Rana et al. noted that pre-eclampsia is a proinflammatory state, but the culpable cells have yet to be fully elucidated.9 Syncytial knots are allogenic nano to microvesicles shed from apoptotic or activated trophoblasts that have been identified in the lungs and plasma of normal pregnancies and in increased amounts in pre-eclampsia.9 Rich in sFLT1 and endoglin, syncytiotrophoblast microvesicles and exosomes may instigate an inflammatory response.9 Proinflammatory cytokines include TNFα, MIP-1α, IL-1α, IL-1β, IL-6, and IL-8. In vitro, syncytiotrophoblast microvesicles activate cultured peripheral blood mononuclear cells, causing a release of proinflammatory cytokines. that is even more robust when exposed to peripheral blood mononuclear cells from pregnant patients.9

IL (Interleukin)-10, a cytokine that induces the differentiation of the T cell into the Th (T helper type)2 phenotype, is an important mitigator of the maternal syndrome by neutralizing proinflammatory cytokines, AT1-AA (angiotensin II receptor 1 autoantibodies), placental ROS, and ET-1 (endothelin-1).9 Many cell types in pre-eclamptic patients demonstrate a dysregulation in the balance of IL-10 and proinflammatory cytokines, including uterine and circulating NKs and peripheral blood mononuclear cells.9 Peripheral blood mononuclear cells of pre-eclamptic women had reduced IL-10 secretion, which may lead to a failure of T-cell differentiation. Normal pregnancy is characterized by a shift in T-cell phenotype towards Th2 relative to Th1 and an aberrant shift towards the Th1 phenotype has been reported in pre-eclampsia, resulting in insufficient trophoblast invasion.9

(iv) Elevated complement levels with hemolysis, elevated liver enzymes, and low platelets: Pre-eclampsia is also associated with elevated complement levels and with genetic mutations in C3.9 In animal models, complement inhibition restores spiral artery capacitance and decreases sFLT1 production, and a C1q knockout mouse
model mimics pre-eclamptic features.\(^9\) However, complement dysregulation is most severe in the form of severe pre-eclampsia called hemolysis elevated liver enzymes low platelets (HELLP) syndrome. HELLP syndrome has been shown to share a genetic mutation with atypical hemolytic uremic syndrome, a disease thought to be caused by uncontrolled complement activation, and has a similar presentation.\(^9\) Many of the same complement pathway mutations found in hemolytic uremic syndrome are also associated with pre-eclampsia.\(^9\)

(v) Increased angiotensin II sensitivity including hypersensitivity of the angiotensin II type 1 receptor (AT1) through forming a complex with the bradykinin B2 receptor: The renin-angiotensin-aldosterone system is also involved in the pathogenesis of pre-eclampsia with angiotensin II sensitivity being enhanced, compared to a normal pregnancy, both before the onset of pre-eclampsia and during pre-eclampsia, despite there being reduced levels of circulating renin and angiotensin II during pre-eclampsia.\(^9\) One potential mechanism for the increased angiotensin II sensitivity is the presence of circulating autoantibodies to angiotensin II type 1 receptor (AT1) in the sera of pre-eclamptic women.\(^9\) In preclinical studies, autoantibodies to AT1 reproduce many of the hallmark characteristics of pre-eclampsia: (i) vasoconstriction through activation of ET-1; (ii) endothelial cell necrosis and apoptosis in human umbilical vein endothelial cells; (iii) stimulation of tissue factor production contributing to hypercoagulation; (iv) reduction of trophoblast invasion in human cell culture models; and (v) increased production of ROS in culture models.\(^9\) Produced in response to placental ischemia and systemic inflammation, anti-AT1-AA can also stimulate placental production of antiangiogenic factors sFLT1 and sENG.\(^9\) In addition, CD19+CD5+ cells, as well as antiangiotensin II type I receptor autoantibodies (anti-AT1-AA) activity, are elevated in the sera of pre-eclamptic patients, suggesting that B lymphocytes are involved in the immune reaction in pre-eclampsia.\(^9\) These findings suggest that anti-AT1-AA made by a subpopulation of CD19+CD5+ in response to placental ischemia and systemic inflammation may contribute to the hypertension and production of antiangiogenic factors that characterize the maternal syndrome.\(^9\)

The renin-angiotensin-aldosterone system may also be activated, in the setting of downregulated renin, by the hypersensitivity of the AT1 receptor being increased through forming a complex with the bradykinin B2 receptor.\(^9\) Elevated levels of an oxidized form of angiotensinogen that is more readily cleaved by renin have also been implicated in the pathogenesis of the hypertension observed in pre-eclampsia.\(^9\) In animal models, elevated levels of circulating sFLT1 were sufficient to induce angiotensin II sensitivity by interfering with endothelial nitric oxide production.\(^9\)

(vi) Increased sympathetic nervous system activity: The sympathetic nervous system may also be involved in the pathogenesis of pre-eclampsia as muscle sympathetic nerve activity is elevated in women with pre-eclampsia compared to normal pregnant and hypertensive women, and nonpregnant control women.\(^9\) Women with pre-eclampsia also have reduced baroreflex sensitivity and greater antihypertensive responses to nonselective adrenergic receptor blockade.\(^9\)

(vii) Vitamin D deficiency: In a 2013 systematic review and meta-analysis of nine observational studies, Aghajafari et al. found that pregnant women with pre-eclampsia had significantly lower concentrations of 25-hydroxyvitamin D than the
comparison group (pooled weighted mean difference –14.53 nmol/L, 95% CI –22.57 to –6.49). All the studies reporting on means and standard deviations were of case-control design.

**POSSIBLE MECHANISMS WHEREBY FLUORIDE MIGHT AFFECT THE PATHOPHYSIOLOGY OF PRE-ECLAMPSIA:**

Many mechanisms have been proposed for the pathophysiology of pre-eclampsia and it is likely that at least some of them will be affected by fluoride. Fluoride is an enzyme poison, inducing oxidative stress, hormonal disruptions, and neurotoxicity. Fluoride in synergy with aluminium acts as a false signal in G protein cascades of hormonal and neuronal regulations in much lower concentrations that fluoride acting alone. At the cellular level, fluoride: (i) inhibits enzymes through competition with magnesium; (ii) inhibits the phosphoryl transfer reaction; (iii) inhibits adenosine triphosphatases by multifactorial mechanisms; (iv) acts as an enzyme disruptor with many enzymes from a variety of tissues with both inhibitory and stimulatory effects; and (v) forms aluminofluoride complexes which disrupt transmembrane signalling, G protein signalling cascades, and integrative networks.

By considering the current views on the pathophysiology of pre-eclampsia and the known effects of fluoride, the following are possible mechanisms whereby fluoride might affect the pathophysiology of pre-eclampsia.

(i) **Possible effect of fluoride on the impaired cytotrophoblast transformation from the proliferative epithelial subtype to the invasive epithelial subtype:** Thyroid hormones are necessary for the proper functioning of all tissues and are particularly needed for tissue transformation such as metamorphosis in amphibians and placental development in vertebrates with cytotrophoblast transformation from the proliferative epithelial subtype to the invasive epithelial subtype. The inhibition of metamorphosis, the reduced head-tail length, and the disturbed hard tissue ossification in tadpoles with exposure to fluoride ions can be described as examples of fluoride-induced developmental disorders (FIDD). FIDD may be considered to be disorders in which fluoride ions have disrupted, with arrest, reduction, delay, or acceleration, the normal development, growth, or maturation of an organism, at some stage between the commencement of the life of the organism and its becoming an adult. After considering (i) the ability of fluoride, in amphibians, to inhibit metamorphosis and growth, and to disturb hard tissue ossification; (ii) the control of amphibian metamorphosis by triiodothyronine (T3) and the sonic hedgehog signalling pathway; (iii) human fluoride-induced developmental disorders (FIDD) with short stature, bone deformities, cognitive impairment, delayed dental eruption, and dental fluorosis; (iv) the effects of fluoride on thyroid hormone metabolism and the sonic hedgehog pathway which result in a decrease in the serum T3 and an elevation in the serum rT3; and (v) the evidence linking fluoride exposure, thyroid hormone metabolism, the sonic hedgehog signalling pathway, and the listed human FIDD; it has been concluded that these amphibian and human FIDD can be considered to result from disturbed thyroid hormone metabolism and sonic hedgehog signalling.

Low levels of fluoride exposure can be sufficient to result in FIDD and the threshold for the drinking water fluoride level for the development of fluoride-
induced neurotoxicity was calculated to be approximately 0.2 mg/L by Hirzy et al.\textsuperscript{14} in 2016 and Grandjean\textsuperscript{15} in 2019.\textsuperscript{16} However the timing of the fluoride exposure is also critical and the developing brain is most sensitive to fluoride-induced neurotoxicity during the intrauterine period.\textsuperscript{17} The developing brain may also be adversely affected during infancy by exposure to drinking water with a fluoride level of approximately 0.6 mg/L.\textsuperscript{16,18} The inadequate placental development in pre-eclampsia resulting from the impaired cytotrophoblast transformation from the proliferative epithelial subtype to the invasive epithelial subtype can be seen as a further example of a fluoride-induced developmental disorder and a possible mechanism whereby fluoride be causally related to pre-eclampsia.

\textit{(ii) Atherosclerosis of the uterine spiral and radial arteries:} Oxidative stress is a risk factor for atherosclerosis\textsuperscript{19} and fluoride is able to produce oxidative stress.\textsuperscript{11,12} The pathological characteristics of atherosclerosis include lipid accumulation, fibrosis formation, and the production of atherosclerotic plaque in the arterial intima, leading to vascular sclerosis, lumen stenosis, and ischemic changes of corresponding organs. Atherosclerosis is closely associated with endothelial dysfunction. Oxidative stress describes an imbalance state where the production of reactive oxygen species (ROS), including superoxide (O$_2^-$), hydrogen peroxide (H$_2$O$_2$), and hydroxyl radicals (OH) exceeds antioxidant defences.\textsuperscript{20} Several enzyme systems contributing to the formation of ROS, including NAD(P)H oxidase, xanthine oxidase, and mitochondrial electron leakage from electron transport chain.\textsuperscript{20} ROS are normally generated as a natural byproduct of oxygen metabolism and play important roles in cell signaling but their level can be markedly increased under oxidative stress conditions, such as heart failure, ischemia-reperfusion and aging.\textsuperscript{20} Nitric oxide (NO) is a multifunctional signaling molecule and potent endogenous vasodilator which is involved in the maintenance of metabolic and cardiovascular homeostasis and the suppression of the key processes leading to the development of atherosclerosis.\textsuperscript{19} Oxidative stress can reduce NO bioavailability and contribute to atherosclerosis by causing endothelial dysfunction with lipid infiltration, the expression of some inflammatory factors, an alteration of vascular tone, an enhancement of arginase activity, an increase in asymmetric dimethylarginine, and hyperhomocysteinemia.\textsuperscript{19} Other factors in the aetiology of atherosclerosis which also influence NO bioavailability include diabetes mellitus, obesity, chronic kidney disease, and smoking.\textsuperscript{19}

However, Stanhewicz and Alexander considered in 2017, on the basis of \textit{ex vivo} analysis of cutaneous tissue samples and \textit{in vivo} cutaneous vasodilator responsiveness, using intradermal microdialysis fibres, to exogenous acetylcholine, local heat, angiotensin II, and norepinephrine, in 24 healthy, normotensive, postpartum women who had delivered in the past 12 months, and of whom 12 had a history of pre-eclampsia, that women who have had pre-eclampsia do not have attenuated NO production and that the NO is reaching the vascular smooth muscle.\textsuperscript{21} They concluded that mechanisms secondary to NO production and diffusion to the vascular smooth muscle, such as increased vasoconstrictor tone, are affecting both endothelium- and NO-dependent dilation in the women with a history of pre-eclampsia.\textsuperscript{21}
Stanhewicz and Alexander noted that vasoconstrictor sensitivity to circulating angiotensin II is attenuated in normal pregnancy and this reduced sensitivity contributes to the normal reduction in systemic vascular resistance observed in healthy pregnancy. In contrast, women who develop pre-eclampsia have an exaggerated pressor response to angiotensin II during pregnancy, an effect that is likely mediated by increased circulating inflammatory cytokines and concomitant increases in circulating agonistic antibodies to the angiotensin II type 1 receptor (AT1-AA). Similarly, previously pre-eclamptic women demonstrated an augmented pressor response to systemic angiotensin II infusion post-partum, suggesting that this augmented sensitivity that arose during pregnancy persisted post-partum. Stanhewicz and Alexander demonstrated that women who have had pre-eclampsia exhibit a greater microvascular vasoconstrictor response to angiotensin II than control women matched for age and time post-partum. The lack of difference in norepinephrine-mediated vasoconstriction between the groups suggested that the increased sensitivity to angiotensin II observed in formerly pre-eclamptic women was specific to the angiotensin II-activated receptor and signalling mechanisms, and not generalized to receptor mediated vasoconstrictor stimuli. In addition, ex vivo analysis showed that women who had pre-eclampsia had increased angiotensin II type 1 receptor (AT1R) protein expression and that it was likely that this increased AT1R expression contributed to the increased sensitivity to angiotensin II observed in these women. The authors also found that local administration of the AT1R inhibitor losartan augmented endothelium-dependent and NO-dependent dilation in formerly pre-eclamptic women but had no effect in women with a history of healthy pregnancy. This gave further support to augmented angiotensin II sensitivity contributing to the persistent microvascular dysfunction in women who have had pre-eclampsia.

Stanhewicz and Alexander observed that while circulating angiotensin II is not elevated during- or post-pregnancy in women with pre-eclampsia, the AT1-AA concentrations increase in women during a pre-eclamptic pregnancy and remain elevated post-partum. They considered that it was likely that chronically elevated AT1-AA contributed to the persistent endothelial dysfunction in women who had had pre-eclampsia, independent of endogenous angiotensin II concentrations.

(iv) Impaired stromal transformation of uterine endometrium to prepare for implantation: As noted, thyroid hormones are necessary for the proper functioning of all tissues and are particularly needed for tissue transformation, such as the stromal transformation of the uterine endometrium to prepare for implantation, and that fluoride can disrupt thyroid hormone metabolism.

(v) Overexpression of hypoxia-inducible transcription factors HIF-1α: and HIF-2α: The cellular responses to hypoxia are complicated because they involve both transcriptional and post-transcriptional steps as well as pre-mRNA splicing and oxygen tension-dependent alternative splicing. As fluoride can inhibit and stimulate enzymes, inhibit the phosphoryl transfer reaction, inhibit adenosine triphosphatases, act as an enzyme disruptor with many enzymes from a variety of tissues with both inhibitory and form aluminofluoride complexes which disrupt transmembrane signalling, G protein signalling cascades, and integrative networks, it...
is likely that fluoride will have some effect on the expression of hypoxia-inducible transcription factors HIF-1α: and HIF-2α.\textsuperscript{11}

(vi) Oxidative stress with an imbalance of reactive oxygen species (ROS)-generating enzymes and antioxidants from mitochondrial stress and endoplasmic reticulum stress: Fluoride can produce both mitochondrial stress and endoplasmic reticulum stress. Quadri et al. noted that reactive oxygen species and lipid peroxidation have been considered to play an important role in the pathogenesis of chronic fluoride ion toxicity.\textsuperscript{23} Many studies have shown that F-induced apoptosis may be associated with oxidative stress and provoke the initiation of the apoptotic process.\textsuperscript{23} Apoptosis is mainly regulated by complex pro- and anti-apoptotic gene families.\textsuperscript{23} The Bcl-2 family of regulator proteins has been demonstrated to be involved in the regulation of apoptosis with Bcl-2 inhibiting apoptosis and Bax promoting it.\textsuperscript{23} Both genes are widely present in the mitochondria and endoplasmic reticulum of the cells. Quadri et al. evaluated fluoride-associated mitochondriopathy in human renal cells by studying, using transmission electron microscopy, renal biopsy from 64 children, aged 4–12 yr, with nephrotic syndrome due to minimal change disease with either a normal urinary fluoride level (≤1 mg/L) or a high urinary fluoride level (>1 mg/L).\textsuperscript{23} The patients in the high urinary fluoride group showed a remarkably high level of mitochondriopathy including mitochondrial edema, cristolysis, and electron dense deposits in the mitochondrial matrix.\textsuperscript{23}

Izquierdo-Vega et al. studied the effect of environmentally relevant doses of fluoride on \textit{in vitro} fertilization (IVF) capacity of spermatozoa, and its relationship to spermatozoa mitochondrial transmembrane potential [DeltaPsi(m)] in male Wistar rats who were administered 5 mg fluoride/kg body mass/24 hr, or deionized water orally for 8 weeks.\textsuperscript{24} The spermatozoa from the fluoride-treated rats exhibited a significant decrease in superoxide dismutase (SOD) activity (~33%), accompanied with a significant increase in the generation of superoxide anion O$_2^-$ (~40%), a significant decrease in DeltaPsi(m) (~33%), and a significant increase in lipid peroxidation concentration (~50%), relative to spermatozoa from the control group.\textsuperscript{24} The observations suggested that subchronic exposure to fluoride causes oxidative stress damage and loss of mitochondrial transmembrane potential, resulting in reduced fertility.\textsuperscript{24}

Singh and Das noted that although the exact mechanism of fluoride-induced pathogenesis is not clear, the role of free radicals and lipid peroxidation has been well established by various studies.\textsuperscript{25} The highly toxic lipid peroxidation end-product, 4-hydroxynonenal (HNE), is a nonprotein mediator of apoptosis and has been found to be involved in the pathogenesis of many diseases.\textsuperscript{25} HNE binds with proteins and forms HNE-protein adducts.\textsuperscript{25} These adducts (products resulting from the direct addition of two or more distinct molecules) accumulate in the cellular compartments, especially the endoplasmic reticulum, leading to the disruption of various physiological functions.\textsuperscript{25} Fluoride is known to cause lipid peroxidation and endoplasmic reticulum stress, and they hypothesized that fluoride-induced lipid peroxidation end products may bind with proteins and, in turn, induce abnormalities in cellular function.\textsuperscript{25} They localized the HNE modified proteins in fluoride-exposed cells using immunogold labelling for transmission electron microscopy.\textsuperscript{25} Dietary antioxidants are recommended as a part of the recovery management programs for
fluorosis and they co-treated the cells with dietary antioxidants, namely ascorbic acid (vitamin C) and \( \alpha \)-tocopherol (vitamin E), to investigate the protective role of these antioxidants.\(^{25}\) They confirmed the abundant presence of HNE conjugated proteins in the fluoride-treated cells and that co-treatment with the combination of the dietary antioxidants vitamins C and E was effective in protecting the cells and reducing the fluoride-induced burden of HNE modified proteins in the cells.\(^{25}\)

It is plausible that fluoride may cause oxidative stress with an imbalance of reactive oxygen species (ROS)-generating enzymes and antioxidants from mitochondrial stress and endoplasmic reticulum stress.

(vii) Reduced expression of heme oxygenase-1: Heme oxygenases (HOs) are a family of enzymes involved in the selective catabolism of free circulating heme. While HO-2 is constitutively expressed, HO-1 is strongly overexpressed under stressful stimuli (e.g., oxidative stress). Under these conditions, HO-1 exerts its strong cytoprotective activities and plays a crucial role in stimulating cell survival by removing the pro-oxidant heme and by producing carbon monoxide and biliverdin (promptly reduced to bilirubin).\(^{26}\) As fluoride can inhibit and stimulate enzymes, inhibit the phosphoryl transfer reaction, inhibit adenosine triphosphatases, act as an enzyme disruptor with many enzymes from a variety of tissues with both inhibitory and form aluminofluoride complexes which disrupt transmembrane signalling, G protein signalling cascades, and integrative networks, it is likely that fluoride will have some effect on the expression of heme oxygenases.\(^{11}\)

(viii) Inhibition of the uterine natural killer (NK) cell response: Zhao et al. noted that immunocytes, a class of cells involved in the immune response, include lymphocytes (T lymphocytes, B lymphocytes, K cells, and NK cells), accessory cells (mononuclear phagocytes and dendritic cells), and other immune cells like granulocytes.\(^{27}\) With the exception of the T lymphocytes, whose stem cells mature in the thymus, the other immunocytes originate and mature in the bone marrow. As an irreplaceable primary lymphoid organ, bone marrow replenishes the immune system using a pool of hematopoietic stem cells, and it is a critical site for the sustained production of common lymphoid progenitor cells. It is well known that bone marrow serves as the primary site for development of B cells, NK cells, monocytes, and granulocytes, and that the thymus is the site that provides the main venue for the development and education of T cell progenitors.\(^{27}\) However, accumulated evidence also suggests that the bone marrow can also function as a secondary lymphoid organ for CD4 and CD8 cells, as well as a preferential homing site for memory T cells. Damage to the two primary lymphoid organs can reduce the production of immunocytes and induce immune dysfunction.\(^{27}\) The authors found that, in female rats, excessive fluoride induced thymus apoptosis, reduced weight gain, and induced ultrastructural changes and DNA damage in the immunocytes. Compared to the control group, in the fluoride group, in the thymus, bone marrow, and blood lymphocytes, typical comet configurations were present and the ratio of tailing and the tail length of the immunocytes were significantly increased. Decreases occurred in the blood in the total white blood cell count (p<0.01), lymphocytes (p<0.05), middle cells (p<0.05), granulocytes (p<0.05) and the thrombocytocrit (p<0.05). They concluded that, in female mice, excessive fluoride reduces weight gain, seriously damages the DNA and ultrastructure of immunocytes, and reduces blood total white
blood count, lymphocytes, middle cells, granulocytes, and the thrombocytocrit. It is plausible that fluoride may inhibit the uterine NK cell response.

(ix) Increased expression of the antiangiogenic proteins soluble fms-like tyrosine kinase-1 (sFLT1) and soluble endoglin (sENG): Chang et al. noted that exosomes, membrane-encapsulated vesicles that are released into the extracellular environment by many cell types, can carry signals to the recipient cells to affect inflammation, apoptosis, and angiogenesis. By studying plasma samples from gestational age-matched pre-eclampsia and normal pregnancies they found that exosomes from pre-eclampsia expressed abundant sFlt-1 (soluble fms-like tyrosine kinase-1) and sEng (soluble endoglin). They considered the possibility that extracellular sFlt and sEng were horizontally transferred to human umbilical vein endothelial cells and successfully collected exosomes containing high levels of sFlt-1 and sEng by overexpressing them in human embryonic kidney cells. They found that these exosomes could attenuate the proliferation, migration, and tube formation of human umbilical vein endothelial cells in vitro. In a mouse model, exosomes from pre-eclampsia patients caused vascular dysfunction directly resulted in adverse pre-eclampsia-like birth outcomes. They proposed that exosomes mediated an efficient transfer of sFlt-1 and sEng to endothelial cells to damage vascular functions and induce complications in pre-eclampsia patients. As fluoride can inhibit and stimulate enzymes, including being able to stimulate tyrosine kinase, inhibit the phosphoryl transfer reaction, inhibit adenosine triphosphatases, act as an enzyme disruptor with many enzymes from a variety of tissues with both inhibitory and form aluminofluoride complexes which disrupt transmembrane signalling, G protein signalling cascades, and integrative networks, it is likely that fluoride will have some effect on expression the expression of the antiangiogenic proteins sFLT1 and sEng.11

(xi) Reduced expression of interleukin 10: Kuang et al. examined the suppressive effects of sodium fluoride on cultured splenic lymphocytes in mice. CD3+ T lymphocytes, CD19+ B lymphocytes, cytokines, and cell-cycle markers were analyzed through the use of a cell-counting kit, western blot, and flow cytometry. Splenic lymphocytes were isolated from 3-week-old male ICR mice and exposed to sodium fluoride (0, 100, 500, and 1000 µmol/L) for 24 hr. The percentages of CD3+, CD3+CD4+, CD3+CD8+ T lymphocytes and CD19+ B lymphocytes were decreased (p<0.05 or p<0.01) in the sodium fluoride-exposed cells. This finding was correlated with the alterations in expression levels of cytokine proteins and with evidence of cell-cycle arrest. Thus, protein expression levels of IL-2, TNF-α, IFN-γ, TGF-β were decreased (p<0.05 or p<0.01), and IL-10 protein expression levels were increased (p<0.05 or p<0.01). The percentage of lymphocyte in G1 phase was significantly increased (p<0.05 or p<0.01), while expression levels of cyclin E/D and CDK2/4 were markedly decreased (p<0.05 or p<0.01). These findings demonstrated that sodium fluoride exposure suppressed splenic lymphocyte proliferation, which was represented by reducing populations and activation of splenic T and B lymphocytes. Alterations of cytokine protein expression and cell cycle arrest are the molecular basis of the sodium fluoride-suppressed splenic lymphocyte proliferation, while reduction of T lymphocytes and B lymphocytes is the explanation of sodium fluoride-decreased splenic immune function in vitro.
Southcombe et al. noted that immune adaptation is a critical component of successful pregnancy and that of primary importance is the modification of cytokine production upon immune activation.\textsuperscript{29} With the discovery that normal pregnancy itself is a pro-inflammatory state, it was recognised that the classical Th1/Th2 cytokine paradigm, with a shift towards “type 2” cytokine production, e.g., interleukin-4 (IL-4) and interleukin-6 (IL-6), which are important for antibody production, and away from “type 1” immunity, e.g., interferon gamma (IFNγ) and tumor necrosis factor alpha (TNFα) which are associated with cell mediated immunity and graft rejection, was too simplistic. It is now generally agreed that both arms of cytokine immunity are activated, but with a bias towards “type 2” immunity.\textsuperscript{29} Many factors are released from the placenta that can influence the maternal cytokine balance including syncytiotrophoblast microvesicles (STBM) which are shed from the placenta into the maternal circulation.\textsuperscript{29} STBM can bind to monocytes and B cells and induce cytokine release (TNFα, MIP-1α, IL-1α, IL-1β, IL-6, and IL-8).\textsuperscript{29} Other cytokines are down-modulated, such as interferon-γ (IFNγ)-induced protein 10 (IP-10) which is associated with “type 1” immunity.\textsuperscript{29} Therefore STBM may aid the “type 2” skewed nature of normal pregnancy.\textsuperscript{29} The authors also observed that peripheral blood mononuclear cells (PBMCs: any peripheral blood cell with a round nucleus, e.g., lymphocytes, such as T cells, B cells, and NK cells, and monocytes, in contrast to erythrocytes and platelets which have no nuclei and granulocytes, such as neutrophils, basophils, and eosinophils, which have multi-lobed nuclei) from third trimester normal pregnant women produce more TNFα and IL-6 in response to STBM than PBMC from non-pregnant women, confirming that maternal immune cells are primed by pregnancy, possibly through their interaction with STBM.\textsuperscript{29}

IL (Interleukin)-10, a cytokine that induces the differentiation of the T cell into the Th (T helper type)2 phenotype, is an important mitigator of the maternal syndrome by neutralizing proinflammatory cytokines, AT1-AA (angiotensin II receptor 1 autoantibodies), placental ROS, and ET-1 (endothelin-1).\textsuperscript{9} Many cell types in pre-eclamptic patients demonstrate a dysregulation in the balance of IL-10 and proinflammatory cytokines, including uterine and circulating NKs and peripheral blood mononuclear cells.\textsuperscript{9} Studies of peripheral blood mononuclear cells of pre-eclamptic women had reduced IL-10 secretion, which may lead to failure of T-cell differentiation. Normal pregnancy is characterized by a shift in T-cell phenotype towards Th2 relative to Th1 and an aberrant shift towards the Th1 phenotype has been reported in pre-eclampsia, resulting in insufficient trophoblast invasion.\textsuperscript{9} In contrast to the decrease in IL-10 found in pre-eclampsia, Kuang et al. found the IL-10 protein expression levels were increased by fluoride in cultured splenic lymphocytes in mice.\textsuperscript{28} However, it was considered that fluoride could decrease splenic immune function with a reduction of T lymphocytes and B lymphocytes and the relationship of fluoride to cytokine balance may be complex.

**(xii) Elevated complement levels with hemolysis, elevated liver enzymes, and low platelets:** Pre-eclampsia is associated with elevated complement levels and complement dysregulation is most severe in the HELLP syndrome form of severe pre-eclampsia with hemolysis, elevated liver enzymes, and low platelets.\textsuperscript{9} HELLP syndrome shares a genetic mutation with atypical hemolytic uremic syndrome, a
disease thought to be caused by uncontrolled complement activation, and has a similar presentation.9 Many of the same complement pathway mutations found in hemolytic uremic syndrome are also associated with pre-eclampsia.9 Atypical hemolytic uremic syndrome and thrombotic thrombocytopenic purpura have traditionally been considered separate entities.30 Defects in the regulation of the complement alternative pathway occur in atypical hemolytic uremic syndrome, and defects in the cleavage of von Willebrand factor (VWF)-multimers arise in thrombotic thrombocytopenic purpura.30 However, recent studies suggest that both entities are related as defects in the disease-causing pathways overlap or show functional interactions.30 Noone et al. investigated the possible functional link of VWF-multimers and the complement system on endothelial cells.30 Blood outgrowth endothelial cells (BOECs) were obtained from 3 healthy individuals and 2 patients with Type 3 von Willebrand disease lacking VWF.30 Cells were exposed to a standardized complement challenge via the combination of classical and alternative pathway activation and 50% normal human serum resulting in complement fixation to the endothelial surface.30 The authors found the expected release of VWF-multimers causing platelet adhesion onto BOECs from healthy individuals.30 In the BOECs derived from patients with von Willebrand disease complement C3c deposition and cytotoxicity were more pronounced than on BOECs derived from normal individuals.30 This is of particular importance as primary glomerular endothelial cells display a heterogeneous expression pattern of VWF with overall reduced VWF abundance.30 The results supported a mechanistic link between VWF-multimers and the complement system.30 The findings also identified VWF as a new complement regulator on vascular endothelial cells and suggested that VWF has a protective effect on endothelial cells and complement-mediated injury.30

VWF is synthesized exclusively by endothelial cells, megakaryocytes, and platelets.31 It is stored in specialized secretory granules: Weibel-Palade bodies in endothelial cells and α-granules in platelets.31 The main function of VWF is to initiate platelet adhesion upon vascular injury.31 The hallmark of acute and chronic inflammation is the widespread activation of endothelial cells which provokes excessive VWF secretion from the endothelial cell storage pool.31 The level of VWF in blood not only reflects the state of endothelial activation early on in the pathogenesis but also predicts disease outcome.31 Elevation in the blood level of VWF occurs either by a pathologic increase in the rate of basal VWF secretion or by an increased evoked VWF release from dysfunctional/activated endothelial cells.31 The increase in plasma VWF is predictive of prothrombotic complications and multi-organ system failure associated with reduced survival in the context of severe inflammatory response syndrome, type II diabetes mellitus, stroke and other inflammatory cardiovascular disease states.31 Under physiological conditions, VWF is secreted from endothelial cells via two pathways that enable hemostasis; a continuous or basal secretory pathway that maintains a baseline blood VWF level, and the other, a regulated secretory pathway induced by agonists such as thrombin.31

Once secreted from Weibel-Palade bodies, VWF circulates in a globular conformation under resting conditions and is a carrier for circulating coagulation factor VIII, thereby protecting it from degradation.31 The blood VWF level in health and inflammatory disease is predominantly endothelial cell derived, while the
contribution of platelets is rather minimal. In response to high shear stress and inflammatory mediators, normally quiescent endothelial cells secrete long VWF multimers in large quantities from the storage pool into systemic circulation. VWF multimers are then extracellularly cleaved by a metalloprotease ADAMTS-13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13), unfold in the circulation, and self-associate into particularly linked concatemers to form ultra-large VWF strings. Platelets spontaneously bind to activated VWF via glycoprotein Ibα (GpIbα) interaction with the exposed A1 domain, initiating the thrombogenic process.

Microvascular thrombosis appears to be a major pathological mechanism in sepsis pathology resulting in multi-organ dysfunction syndrome. Hence, sepsis-induced VWF secretion in excess in disseminated intravascular coagulation and multi-organ dysfunction syndrome are inevitably linked. In addition, sepsis-induced ultra-large high molecular weight multimeric VWF permits complement activation, leading to a positive feedback cycle of inflammation and thrombosis. Excessive levels of the highly prothrombotic and multimeric form of VWF and/or ADAMTS13 deficiency constitute a unifying pathologic mechanism linking inflammation to thrombosis.

Hypothyroidism, which may be induced by high levels of fluoride, is associated with a 15% decrease in VWF level. VWF is a surrogate marker of endothelial dysfunction. Upon activation of endothelial cells, VWF acts as an acute phase reactant and correlates with serum C-reactive protein, another acute phase reactant. Oxidative stress can be induced by fluoride and lead to endothelial cell dysfunction and increased VWF levels. The interactions between thyroid dysfunction, oxidative stress, endothelial cell dysfunction, VWF secretion, complement deposition, and cytotoxicity suggest that it is plausible that fluoride may be a factor in the occurrence in pre-eclampsia with elevated complement levels, hemolysis, elevated liver enzymes, and low platelets.

(xiii) Increased angiotensin II sensitivity including hypersensitivity of the angiotensin II type 1 receptor (AT1) through forming a complex with the bradykinin B2 receptor: Angiotensin II sensitivity is enhanced, compared to a normal pregnancy, both before the onset of pre-eclampsia and during pre-eclampsia, despite there being reduced levels of circulating renin and angiotensin II during pre-eclampsia. One potential mechanism for the increased angiotensin II sensitivity is the presence of circulating autoantibodies to angiotensin II type 1 receptor (AT1) (anti-AT1-AA) in the sera of pre-eclamptic women. Anti-AT1-AA are produced in response to placental ischemia and systemic inflammation. Anti-AT1-AA can also stimulate placental production of antiangiogenic factors sFLT1 and sENG. In addition, CD19+CD5+ cells, as well as antiangiotensin II type 1 receptor autoantibodies (anti-AT1-AA) activity, are elevated in the sera of pre-eclamptic patients, suggesting that B lymphocytes are involved in the immune reaction in pre-eclampsia. These findings suggest that anti-AT1-AA, made by a subpopulation of CD19+CD5+ in response to placental ischemia and systemic inflammation, may contribute to the hypertension and production of antiangiogenic factors that characterize the maternal syndrome. As well as fluoride being able to inhibit and stimulate enzymes, inhibit the phosphoryl transfer reaction, inhibit adenosine triphosphatases, act as an enzyme disruptor with many enzymes from a variety of tissues with both inhibitory and form
aluminofluoride complexes which disrupt transmembrane signalling, fluoride can decrease splenic B lymphocytes and it is plausible that fluoride contributes to the increased angiotensin II sensitivity, including hypersensitivity of the angiotensin II type 1 receptor (AT1).

(xiv) Increased sympathetic nervous system activity: The serum of fluoride-treated mice has been reported to show enhanced levels of adrenaline and noradrenaline with the effect being mediated by stress. It is possible that fluoride may contribute to increased sympathetic nervous system activity.

(xv) Vitamin D deficiency. In a 2020 review, Charoenngam and Holick noted that as well as vitamin D being responsible for the regulation of calcium and phosphate metabolism and maintaining a healthy mineralized skeleton it was also an immunomodulatory hormone. They recorded that experimental studies have shown that 1,25-dihydroxyvitamin D, the active form of vitamin D, exerts immunologic activities on multiple components of the innate and adaptive immune system as well as endothelial membrane stability. An association has been shown between low levels of serum 25-hydroxyvitamin D and an increased risk of developing several immune-related diseases and disorders, including psoriasis, type 1 diabetes, multiple sclerosis, rheumatoid arthritis, tuberculosis, sepsis, respiratory infection, and COVID-19, has been observed.

Humans gets vitamin D from sunlight, diet, and supplements. There are two major forms of vitamin D: vitamin D2 and vitamin D3. Vitamin D2 is synthesized from ergosterol and found in yeast, sun dried and ultraviolet irradiated mushrooms, and plants. Vitamin D3 is synthesized endogenously from 7-dehydrocholesterol in the skin and found naturally in cod liver oil and oily fish. After entering the circulation, vitamin D (D represents either or both vitamin D2 and vitamin D3) is metabolized by the vitamin D-25-hydroxylase (CYP2R1) in the liver to 25-hydroxyvitamin D [25(OH)D]. 25(OH)D is further metabolized by the enzyme 25-hydroxyvitamin D-1α-hydroxylase (CYP27B1) to the active form, 1,25-dihydroxyvitamin D [1,25(OH)2D]. 1,25(OH)2D exerts its physiologic functions in the target tissue by binding to the vitamin D receptor (VDR) in the nucleus where it leads to up- or down-regulation of a multitude of genes. Although the main site of conversion of 25(OH)D into the systemically bioavailable 1,25(OH)2D is in the kidneys, CYP27B1 is also expressed by many other tissues including activated macrophages, parathyroid glands, microglia, breast, colon, and keratinocytes where 1,25(OH)2D is produced and exerts its autocrine and paracrine functions.

1,25(OH)2D modulates the differentiation and functions of antigen-presenting cells by inducing them to become more immature and tolerogenic, characterized by a decrease in the expression of major histocompatibility complex (MHC) class II and co-stimulatory molecules on the cell surface. This results in a decrease in antigen presentation and production of interleukin-12 (IL-12), and an increase in production of IL-10, a tolerogenic cytokine. 1,25(OH)2D is also shown to suppress the expression of toll-like receptors on the monocytes and inhibit the production of some inflammatory cytokines such as IL-2, IL-6, and IL-17. In addition, experimental studies have suggested that differentiation and function of natural killer (NK) cells can be modulated by 1,25(OH)2D treatment.
A number of experimental studies have shown that vitamin D and its metabolites modulate endothelial function and vascular permeability via multiple genomic and extra-genomic pathways. A study found that in the primary dermal human microvascular endothelial cell model, vitamin D3, 25(OH)D3 and 1,25(OH)2D3 non-genomically stabilized vascular endothelium, and that vitamin D3, normally circulating at about 100 times higher level than 1,25(OH)2D3, was at least 10 times more potent than 1,25(OH)2D3 and more than thousand times more potent than 25(OH)D3 in stabilizing the endothelium. In addition, 1,25(OH)2D3 is a transcriptional regulator of endothelial nitric oxide synthase (eNOS), causing up-regulation of eNOS gene expression and therefore increased endothelial production of nitric oxide. The effect of 1,25(OH)2D3 on endothelial nitric oxide production occurred most robustly within one minute after administrating the compound, implying that the action of 1,25(OH)2D3 was non-genomic. Activation of VDR by 1,25(OH)2D at the endothelial cell membrane is shown to increase eNOS activity via intracellular second messenger pathways including adenylyl cyclase/cyclic adenosine monophosphate (AC/cAMP) and inositol trisphosphate/diaecylglycerol (IP3/DAG) pathways, which result in increased intracellular calcium concentration. Activation of VDR also activates eNOS via the phosphoinositide 3-kinase/protein kinase b (PI3K/ Akt) pathway that triggers phosphorylation of serine-1779 on eNOS. Furthermore, in a uremic rat model using the immunofluorescence technique, 1,25(OH)2D2 promoted vascular endothelial-cadherin-based cell-cell junctions and inhibited F-actin stress fiber organization, thereby preventing the formation of endothelial intracellular gaps and attenuating endothelial damage in the presence of chronic kidney disease. Taken together, it is evident that vitamin D and its metabolites exert pleiotropic effects on the vascular endothelium that are protective against vascular dysfunction and tissue injury as a result of local and systemic inflammation.

Peripheral blood monocytes express VDR but resting T lymphocytes did not. When resting T lymphocytes were stimulated with phytohemagglutinin, they became activated and with the activation, T cells expressed the VDR. Activated T lymphocytes are known to express CYP27B1 that mediates local conversion of 25(OH)D into 1,25(OH)2D which is believed to stimulate intracrine activation of VDR.

In general, 1,25(OH)2D produced locally by monocytes/macrophages results in a dramatic shift of immune status from proinflammatory state to tolerogenic state. 1,25(OH)2D suppresses the proliferation of T lymphocytes, and modulates cytokine production and differentiation with diverse effects on different subgroups of T lymphocytes. It promotes a shift from TH1 and TH17 to TH2 immune profile by suppressing the expression of TH1 (IL-2, IFN-γ, TNF-α) and TH17 (IL-17, IL-21) cytokines while inducing the expression of TH2 cytokines (IL-4, IL-5, IL-9, IL-13). 1,25(OH)2D can also promote differentiation of regulatory T cells (Treg) both directly and indirectly via its interaction with antigen-presenting cells, resulting in a suppression of proinflammatory state. This is believed to be one of the explanations by which vitamin D could exert protective effects against autoimmune diseases.

Similar to T helper cells, cytotoxic T lymphocytes (CTL) express both CYP27B1 and VDR, and upregulation of VDR can be observed in response to infection as well as mitogen stimulation, suggesting a coordinate regulation of VDR signaling
pathway and CTL responses. Studies have shown that decreased CD4/CD8 ratio, an indicator of increased immune activation, was associated with low levels of 25(OH)D and that giving 5000–10,000 IU of vitamin D3 was associated with an increase in CD4/CD8 ratio, reflecting immune suppression. However, little is known about the direct influence of vitamin D on CTL. The effects of 1,25(OH)2D on differentiation, proliferation, and functions of CTL are likely mediated by both direct intracrine activation of VDR and alteration of cytokines signaling via T helper cells and antigen-presenting cells.

Inactive B lymphocytes have no VDR, and only when they become activated to proliferate by mitogens do they upregulate their VDR expression. Initially, it was found that 1,25(OH)2D inhibited immunoglobulin synthesis and therefore could potentially be detrimental to the immune system. However, a variety of studies have demonstrated that just as 1,25(OH)2D modulates T cell function so too does it regulate B-cell activity. When in a hyperactive state, 1,25(OH)2D appears to dampen the immunoglobulin immune response by a variety of mechanisms. It inhibits plasma cell formation and inducing apoptosis of both activated B cells and plasma cells. It also inhibits cytokine-mediated B-cell activation by acting on T-helper cells, directly promotes B-cell anti-inflammatory cytokines production (IL-10, CCR10) and suppresses the differentiation from mature B cells to plasma cells and class-switched memory B cells. It is believed that, by controlling B-cell activity and B-cell transformation into the plasma cells, 1,25(OH)2D helps reduce autoantibody production, thereby reducing risk for antibody-mediated autoimmune disorders such as systemic lupus erythematosus. It has also been suggested that lymphocytes also have the capacity to generate 1,25(OH)2D. Only B lymphocytes can express CYP27B1 and 25(OH)D in vitro at 25 times the level of 1,25(OH)2D can cause similar biologic responses as 1,25(OH)2D in B lymphocytes. However, there has not been any direct demonstration that B lymphocytes can produce 1,25(OH)2D.

Many of the actions of vitamin D and its metabolites are relevant to the pathophysiology of pre-eclampsia through their effects on immune function. The conversion of vitamin D2 and vitamin D3 to the active forms involves enzymes and G proteins which can be affected by fluoride. It is plausible that fluoride can contribute to vitamin D deficiency.

**Conclusion:** The current knowledge on pre-eclampsia indicates that it is a complex multisystem disease. Central to the pathogenesis are abnormal placentation with uteroplacental ischaemia early in the first trimester and a hypertensive maternal syndrome with multi-organ failure, later in the second and third trimesters, with the release into the maternal circulation from the ischaemic placenta of soluble toxic and antiangiogenic factors, such as soluble fms-like tyrosine kinase (sFLT1) and soluble endoglin (sENG), which result in inflammation, endothelial cell dysfunction, and maternal systemic disease. The placental ischaemia may lead to small for gestational age babies. Various theories have been proposed to explain the condition. To help establish whether or not the relationship between fluoride exposure and pre-eclampsia might be one of causation rather than association, two of the nine Bradford Hill criteria establishing a causal relationship were considered. When coherence was examined with respect to low birth weight, the generally known facts of the natural history and biology of fluorosis, and pre-eclampsia, no serious conflict was found.
The criterion of plausibility was examined by considering whether or not there were known effects of fluoride that might affect the proposed pathophysiology. The result was that it was considered plausible that fluoride might be able to affect the development of the disease. At present, on this basis, it is more likely than not that fluoride is a risk factor for the development of pre-eclampsia. However, the pathophysiology of pre-eclampsia is complex and will become clearer over time as more research is done.

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