# INTRODUCTION

Hypertensive disorders is one of the most frequent gestational medical disorders and the incidence of hypertensive diseases have a range from 5% to 6% in all types of gestations ***(Hauth et al., 2000).***

Hypertensive diseases in conjunction with bleeding and infectious diseases are the corner stone causes for the bulk of maternal morbidity and mortality during gestation. Additionally, hypertension accounts for around 16% of maternal mortalities in developed nations ***(Cunningham et al., 2010).***

The pathophysiological pathways of PET have not been clarified until current time. On the other hand, it is proven by various researchers that PET is featured and displayed at molecular and cellular levels by disproportionate pathological inflammatory reaction ***(Kristensen et al., 2009).***

Together PET and cardiovascular disorders are correlated to the pathophysiological inflammatory pathways and have in common various risk factors e.g obesity. In various priorly performed research studies, dissimilar findings and data have been mentioned for the CRP levels, which is a clinical inflammatory biomarker and described as an acute phase reactant, in PET Gestations ***(Engin-Ustün et al., 2007)***.

An additional inflammatory marker, serum amyloid A, is a precursor form of protein causing the configuration of amyloid A fibrils in systemic amyloid A (AA) amyloidosis and has a cornerstone pathological role in the progress of systemic amyloidosis disease ***(Sandri et al., 2014)***

Serum amyloid A levels rise in a significant manner as pathophysiological response to infectious viral and bacterial insults, tumor enlargement and physical strain ***(Eklund et al., 2012)***.

Serum amyloid A may rise up to 1000-fold and could reach to 500-1000 μg/ mL. Amyloid A is synthesized by hepatic cells as an inflammatory response to cytokines ***(Ferguson et al., 2017; De Buck et al., 2015; Ye and Sun, 2015).***

It has been reported that additionally amyloid A in serum is manufactured by various cell types such as fibroblasts, macrophages and fat cells. Restricted number of research studies have assessed and evaluated amyloid A levels in serum of gestations suffering the development of PET ***(Can et al., 2011; Hansen et al., 2015).***

# Aim of the Study

The current research study aims to investigate and evaluate serum levels of amyloid A in PET and healthy gestations, to help in detection of a correlation between PET and serum levels of amyloid A.

## Research question:

Does serum amyloid A elevated in preeclampsia?

## Research hypothesis:

Serum amyloid A may be elevated in preeclampsia

# Amyloid a Protein in Normal Gestation and Preeclampsia

Preeclampsia is a common complication of gestation and remains a chief cause of maternal and fetal mortality. The clinical symptoms of preeclampsia are caused by widespread endothelial dysfunction proposed to be a part of an exaggerated maternal inflammatory response to pregnancy. The syndrome of preeclampsia is a condition with several manifestations, and clinically suspicious organ impairment is monitored by serum markers for hemolysis, coagulopathy, liver and renal function. In women with preeclampsia, the impairment of renal function is a negative factor used as a key to optimize the time of delivery. The plasma level of cystatin C, a low molecular mass plasma protein, has been proven to be a reliable marker of renal impairment in preeclampsia. This marker is not influenced by inflammation ***(Yan et al., 2014).***

Serum amyloid A (SAA) and C-reactive protein (CRP) are classified acute phase proteins mainly synthesized and released by hepatic cells. Other cells involving lymphocytes, monocytes, and macrophages can similarly produce these proteins. The induction of SAA and CRP synthesis is triggered by a number of cytokines, chiefly IL-6 and TNF- predominantly released from macrophages and monocytes at the inflammatory sites. The

synthesis is influenced by steroid hormones and adipose tissue (due to IL-6 production in the adipocytes). Increased baseline levels of SAA and CRP analyzed by high-sensitivity assays have been recognized as markers of vascular wall inflammation and as clinical markers for the prediction of cardiovascular events ***(Silvana et al., 2014).***

Since preeclampsia is associated with widespread endothelial dysfunction, proposed to be provoked by an increased maternal systemic inflammatory response, the maternal plasma levels of SAA and CRP might be expected to be increased when compared to normal pregnancy levels. The maternal plasma levels of SAA and CRP in normal pregnancy could differ from non-pregnant levels due to increased hormone levels, increased adipose tissue and/or secondary to modifications of the inflammatory response in normal pregnancy ***(Sandri et al., 2014).***

Previous studies have found an elevated CRP level in normal pregnancy compared to non-pregnant levels, and other studies have reported an elevated CRP level in preeclampsia compared to normal pregnancy levels .In two of these research studies, however, the association was mitigated after adjusting for BMI. The level of SAA, the other major acute phase protein, has previously been found to be unaltered by pregnancy. In a pilot research study, the SAA level was revealed to be increased in women with preeclampsia correlating with other proinflammatory cytokines ***(Ye et al., 2015).***

Since the etiology of preeclampsia is still largely mysterious, numerous concepts have been proposed to unravel the mysteries of this clinical condition. There is research obtained evidence that preeclampsia is linked with a widespread endothelial dysfunction proposed to be triggered by an excessive inflammatory response. The absence of normal remodeling of decidual spiral arteries, sometimes seen in preeclampsia, has been associated with local inflammation suggested to be part of the pathogenesis ***(Yan et al., 2014).***

In a recent research study of peripheral endothelial-dependent microvascular function in normal gestation, on the other hand, assayed markers of inflammation and endothelial activation did not appear to explain changes in the microvascular responsiveness ***(Connolly et al., 2011).***

In pregnancy, risk factors for preeclampsia such as obesity, diabetes and hypertensive disorders are associated with pro-coagulatory and proinflammatory disturbances in the endothelium. In inflammation, the endothelium is not only a major target, but also a perpetuator of local inflammation, causing a disturbed regulation of vascular tone and the development of a procoagulatory, proinflammatory, and finally a pro-atherosclerotic vascular environment. Inflammatory mediators e.g CRP and TNF- downregulate the endothelial nitric oxide synthetase

expression levels causing a reduction in nitric oxide production. It is close at hand, then, to regard inflammation also as a risk factor for preeclampsia ***(Silvana et al., 2014).***

Endothelium dysfunction triggered by an inflammatory stimulus, could hypothetically be weakened with free radical scavengers e.g. vitamin C or administration of statins or aspirin. Though, research studies in pregnancy have failed to display any major preventive impact of pharmacological treatment on women at high risk for preeclampsia ***(Sandri et al., 2014).***

Inflammation in normal pregnancy have been suggested by raised plasma levels of IL-6 and TNF- and by activated leukocytes and monocytes revealed and displayed by flow cytometry and in vitro stimulation of peripheral blood mononuclear cells. In preeclampsia, the circulating pro inflammatory cytokines TNF- alpha, IL-6 and IL-8 have been shown to be elevated in comparison to normal pregnancy levels, whether as a cause or a consequence of the condition remains to be elucidated ***(Sandri et al., 2014).***

Cytokines generally exert their biological effect in the picomolar range, and the effects are largely of an autocrine and a paracrine nature. The half-life in vivo generally does not exceed 3 min. Degradation in samples may lead to false low values, whereas false high

concentrations can arise from ex vivo activation. A considerable interassay variation further complicates the interpretation of cytokine concentrations in body fluids. CRP and SAA, the prototypical acute phase proteins, are produced by hepatocytes stimulated by cytokines, particularly IL-6. The baseline CRP is associated with BMI, reflecting the IL-6 production in the adipose tissues. Hepatic synthesis is generally also influenced by steroid hormones ***(Eklund et al., 2012).***

## Serum Amyloid A Levels are raised in Pre-Eclampsia

Pre-eclampsia is a common disorder of pregnancy and a major cause of maternal and fetal morbidity and mortality. Although the exact etiology of pre-eclampsia is not known. An increasing body of evidence supports the existence of a strong link between inflammation, endothelial dysfunction, and pre-eclampsia. However, a cause–effect relationship between these phenomena has not been demonstrated yet. Despite these uncertainties, the relationship between inflammation and pre-eclampsia has prompted the search for markers of inflammation ***(Silvana et al., 2014).***

Increased levels of C-reactive protein (CRP) are associated with the presence and severity of pre-eclampsia.

Increased serum amyloid protein A (SAA), like CRP, has also been shown to reflect systemic inflammation. CRP and SAA are inflammation markers synthesized by hepatocyte upon stimulation by interleukin-6 (IL-6) and tumor necrosis factor alpha, however, both serum levels of SAA and CRP can be increased to about 1,000- fold in response to inflammation ***(Eklund et al., 2012)***.

Pre-eclampsia has been regarded as an inflammatory disease recently. An enhanced inflammatory response and endothelial dysfunction represent important mediating steps in pre-eclampsia. The serum levels of IL-6 and TNF- alpha are higher in the patients with pre-eclampsia than those in normal pregnant woman. SAA is an acute phase protein and apolipoprotein found lesterol transport system. SAA like CRP is an inflammation marker produced in the liver. Their hepatic synthesis is induced by cytokines such as IL-6 and TNF alpha. SAA can affect the onset and progress of endothelial dysfunction by inflammation, however there are no reports on the relation between the SAA and pre-eclampsia ***(Eklund et al., 2012).***

Prior research group of investigators revealed a statistical significant correlations between CRP and SAA. We also investigated the diagnostic performance of SAA in the detection of preeclampsia by using the ROC plot. Additionally they revealed a best cut-off value obtained by

receiver operating analysis was 9.98 ng/l (sensitivity 85%, specificity 75%). The area under the curve was larger for SAA compared with CRP. Therefore, inflammation markers including CRP and SAA are detectable in pre-eclampsia. We believe that pre-eclampsia requires monitoring a panel of multiple inflammatory markers in any single investigation in order to cover all aspects of inflammation reaction ***(Sandri et al., 2014).***

Pre-eclampsia is correlated with an enhanced maternal inflammatory state with oxidative stress that seems to be related to endothelial dysfunction. Some risk factors are known but understanding of the underlying biological mechanisms is limited. Preeclampsia is characterized by poor remodeling of the spiral arteries and there is some evidence pointing to the role of maternal inflammation and oxidative stress. The triggering factor seems to be the placenta, which causes systemic disease characterized by a maternal inflammatory state with oxidative stress ***(Silvana et al., 2014).***

Serum amyloid A (SAA) protein is normally found in the blood in trace amounts; however, it could increase by 1000-fold within 24 h with the effects of inflammation or other stimulants ***(Silvana et al., 2014).***

Serum amyloid A, procalcitonin and TNF alpha are useful biomarkers to understand the maternal inflammatory profile of the disease. As the levels are higher in more severe forms of the disease, these may be beneficial as markers to predict complications such as HELLP and eclampsia to provide the necessary preventive approach in these patients; however, further large scaled studies on this subject are necessary ***(Hansen et al., 2015).***

Serum amyloid A1 (SAA1) is an acute response protein, which is chiefly synthesized by the liver, during the process of infection. On the other hand, it remains unidentified whether SAA1 could be created in human fetal membranes where it is capable to trigger events pertinent to labor commencement. It have been revealed and displayed by research teams that SAA1 is expressed in the fibroblasts and epithelium of the amnion and the trophoblasts of the chorion. Research studies investigating human amnion fibroblasts have shown that SAA1 production is augmented by interleukin-1β (IL-1β) and cortisol alone and synergistically, and SAA1 in turn triggered the expression of IL-1β, interleukin-6 (IL-6), cyclooxygenase-2 (COX-2) and PGE2 production. These impacts of SAA1 are mediated via activation of the NF-κB, p38 and ERK1/2 pathways via the toll-like receptor 4 (TLR4) ***(Wenjiao et al., 2017).***

Inhibition of TLR4 weakened not only SAA1-triggered activation of NF-κB, p38 and ERK1/2 but also raises IL-1β, IL-6 and COX-2 expression levels. Furthermore, SAA1 expressive pattern is raised in human amnion tissue after spontaneous labor. Interestingly SAA1 could be produced in human fetal membranes, which can be greatly induced in the presence of proinflammatory cytokines and glucocorticoids thereby producing effects associated with parturition ***(Wenjiao et al., 2017; Passey et al., 2016).***

Interestingly it was observed that a unique feature of amyloid-like structures is their capacity to induce Thioflavin-T (ThT) fluorescence. Compelling recent research data have displayed that abnormal pathways of protein processing, misfolding and supramolecular aggregations are molecular features present in preeclampsia. Amyloid -like structures circulate in cases with preeclampsia and this may be responsible, at least in partially, for various disease phenotypes and could be used as a disease biomarker. A research group of investigators displayed that amyloid-like aggregates, detected by ThT induced fluorescence, are present in both serum and urine of women with severe preeclampsia and, likely due to their cytotoxic impact, are correlated to clinical severity of HELLP syndrome ***(Katherine et al., 2015).***

# AMYLOID A PROTEIN

**Serum amyloid A** proteins are a group of apolipoproteins correlated with high-density lipoprotein in plasma. Different isoforms of **Serum amyloid A** are expressed at various levels or as a pathological responsiveness to inflammatory triggers (acute phase **Serum amyloid A**). These proteins are synthesized chiefly by the hepatic system ***(Anthony et al., 2014).***

Serum amyloid A proteins were isolated and termed over five decades ago. They are structurally small composed of around 104 amino acids and have a considerable and significant linkage to the acute phase pathological responsiveness with serum levels increasing up to 1000-fold within 24 hours ***(De Beer et al., 2014; Webb et al., 2015).***

Amino-terminal fragments of SAA could form greately organized, insoluble fibrils that collect in secondary amyloid diseases. Although having dynamic synthetic pathway activity SAA proteins have vague physiologic roles. On the other hand considering protein research studies have clarified basic SAA molecular structure and fibril formation. SAA act as a cytokine-like protein that have become known in inter cellular communication in addition to feedback activities in inflammatory ***(Thompson et al., 2015; Sun et al., 2014).***

## Acute-phase serum amyloid A proteins

Acute-phase serum amyloid A proteins are secreted as a response to the acute inflammatory trigger. These proteins have various functions, involving the transportation of cholesterol to the liver to be secreted within the bile, the employment of immune cells to inflammatory zones, and the activation of enzymes that cause degradation of extracellular matrix. Acute-phase serum amyloid A are involved in numerous chronic inflammatory disorders, e.g amyloidosis, atherosclerosis, and rheumatoid arthritis. Three Acute-phase serum amyloid A proteins genes have been revealed and displayed in humans, although the third gene, *SAA3*, is believed to be a pseudogene that does not form messenger RNA or subsequent protein synthesis. Molecular weights of the human proteins are estimated at 11.7 kDa for serum amyloid A type 1 and 12.8 kDa for serum amyloid A type 4 ***(Webb et al., 2015).***

Serum amyloid A is an acute phase bio marker that shows rapid responsiveness similar to CRP, serum levels of acute-phase serum amyloid A rise within hours after an inflammatory trigger, and the amplitude of rise could be greater than that of CRP. Relatively minor inflammatory triggers could cause Serum amyloid A responsiveness at molecular and cellular levels. It has been denoted by

various researchers that amyloid A serum levels correlate better with disease activity in early inflammatory joint disease than do ESR and CRP ***(Gouwy et al., 2015).***

Even though mainly synthesized by hepatocytes, more updated research studies reveal that serum amyloid A is synthesized by adipocytes in addition , and its serum concentration levels is correlated with body mass index ***(Anthony et al., 2014).***

## SAA Genes and Subtypes

To understand the potential functions of SAA in physiologic and pathologic conditions, it is necessary to first examine the SAA genes and subtypes. SAA is the name of a collection of small proteins with 104 AA in their mature forms. These proteins are encoded by 4 separate but closely related genes located on chromosome 11. In humans, the expression of SAA1 and SAA2 is induced by inflammatory cues, including IL-1b, IL-6, and LPS. The induction of these genes involves the transcription factors NF-kB, AP-1, and Yin Yang 1, which are activated during APR. Induced expression of SAA1 and SAA2 by hepatocytes contributes significantly to the rise of SAA in plasma during APR. Human SAA3 is a pseudogene, and human SAA4 encodes a protein that is constitutively produced ***(Den Hartigh et al., 2014; Sun et al., 2014).***