Increased Expression of NLRP3 Inflammasome in Placentas of Gestational Hypertension

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ABSTRACT

Nod-like receptor pyrin 3 is a tripartite protein composed an amino terminal pyrin domain (PYD), nucleotide binding NACHT domain and a carboxy terminal leucinerich repeat (LRR) domain, and consider an intracellular sensor that detects a broad range signal and resulting in the formation and activation the NLRP3 inflammasome. This complex lead to release of the inflammatory cytokines IL-1β, IL-18 which is important for host defense against invading pathogen. Modern evidence indicates that the NLRP3 inflammasome involved in gestational hypertension. This study was conducted on fifty pregnant women and divided into two groups, 25 placentas from normotensive mother having no hypertension before as control group and 25 from gestational hypertension women as gestational hypertension group, obtained from Department of Obstetrics and Gynecology in Almosil El Aam Hospital and Alkanssa Teaching Hospital, Mosul city, Iraq. The placental tissue samples were collected from central region and processed for immunohistochemical technique to expression of NLRP3 in placental tissue. The immunohistochemical study of placenta of gestational hypertension group showed the localization of NLRP3 protein and staining intensity in trophoblast, stroma and endothelium villi were high quantification compared with placenta from control group.

KEYWORDS

NLRP3 inflammasome, Amino terminal pyrin domain, Inflammatory cytokines, Interleukin-1β, Pathogen, Gestational hypertension.
1. Introduction
Reproduction involves tightly regulated series of events and the immune system is involved in an array of reproductive processes. Disruption of well-controlled immune functions leads to infertility, placental inflammation, and numerous pregnancy complications, including gestational hypertension. Inflammasomes are involved in the process of pathogen clearance and sterile inflammation. They are large multi-protein complexes that are located in the cytosol and play key roles in the production of the pivotal inflammatory cytokines, interleukin (IL)-1β and IL-18, and pyroptosis. The nucleotide-binding oligomerization domain, leucine-rich repeat-, and pyrin domain-containing 3 (NLRP3) inflammasome is a key mediator of sterile inflammation activated by various types of damage-associated molecular patterns (DAMPs) (Shirasuna, et al. [1]). The first signal leads to the activation of nuclear factor κB (NF-κB) during different receptors for instance NLRs, TLRs, nuclear factor κB is a transcription factor that interacts with a vast number of genes, and among other things induce expression of pro-IL-1β and NLRP3. These proteins are not constitutively expressed in the preponderance of cells. Hence their concentrations in cells are inadequate to form inflammasomes. The second signal mechanism is not completely delineated. Nevertheless, it appears that it includes the direct binding of DAMPs to NLRP3 (Sutterwala, et al. [2]). Activated NLRP3 multimerize and interact with ASC, which is accountable for the recruitment and activation of procaspase-1 into caspase-1 (Sutterwala, et al. [2], Liu, et al. [3]). Active caspase-1 converts pro-IL1β, pro-IL-18, and gasdermin D (GSDMD) into their active forms (Kelly, et al. [4]). Recent evidence indicates that the NLRP3 inflammasome is involved in gestational hypertension. Thus, the present study was conducted to study the NLRP3 quantification in placenta of gestational hypertension disorders.

2. Materials and Methods
This study was conducted on fifty pregnant women and divided into two group, the first group included 25 pregnant women with gestational hypertension and second group
enrolled 25 pregnant women serves as control group who don’t have gestational hypertension. Fresh placentae were obtained from Department of Obstetrics and Gynecology in Al-Mosul El Aam Hospital and Alkhanssa Teaching Hospital for the period from December 2020 to 1 May 2021 after obtaining the approval from Iraqi Ministry of Health. Exclude placenta from mother with previous hypertension, diabetes, multiple pregnancies. Sample of placenta tissue from gestational hypertension and normal were selected from central region of placenta, Fresh placental tissue pieces were placed in a labelled clean plastic container containing 10% normal buffer saline solution (Yung, et al. [5]). Tissue samples from placentae after delivery were prepared for immunohistochemistry study. Each of tissue samples were usually cut into small parts before another fixation then put the pieces of tissue into embedding cassettes. The water was removed from the parts to be embedded by bathing them consecutively in a graded sequence of mixtures of ethanol and water (70% to 100% ethanol). Ethanol was then replaced with xylene. The tissue was placed in melting paraffin in the oven. The metal embedding model with paraffin were cooling. The hard blocks surrounding the tissues was then taken to a microtome and was sectioned to a thickness of (5 μm). The sections were transferred to glass slides to be stained. Immunohistochemistry staining was prepared according to (Suvarna, et al. [6]). Sections were examined by light microscope with digital camera. Image J analysis software was used to measure quantification of each slide. Statistcal analysis performed by Microsoft Excel (2010) program, values were presented as Mean±SD, and used independent t-test with p – value P≤0.05.

3. Results
Node like receptor pyrin 3 expression was seen to be localized mainly in the trophoblastic cell, stroma, and endothelial cells in placentas of gestational hypertension group (GH) and control group as revealed in Table 1. In the gestational hypertension group, 18(72.00%) cases displayed strong expression of NLRP3 and 7(28.00%) cases registered the medium


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Table (1): NLRP3 reactivity in placenta of control, gestational hypertension groups by using manual microscopic analysis.

<table>
<thead>
<tr>
<th>NLRP3 reactive</th>
<th>Control N=25 n (%)</th>
<th>GH N=25 n (%)</th>
<th>p-value between the groups</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trophoblast</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>20 (80.00)</td>
<td>0 (0.00)</td>
<td>0.005</td>
</tr>
<tr>
<td>Faint</td>
<td>5 (20.00)</td>
<td>0 (0.00)</td>
<td>0.001</td>
</tr>
<tr>
<td>Medium</td>
<td>0 (0.00)</td>
<td>7 (28.00)</td>
<td>0.002</td>
</tr>
<tr>
<td>Strong</td>
<td>0 (0.00)</td>
<td>18 (72.00)</td>
<td>0.004</td>
</tr>
<tr>
<td><strong>Stroma</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>20 (80.00)</td>
<td>0 (0.00)</td>
<td>0.005</td>
</tr>
<tr>
<td>Faint</td>
<td>5 (20.00)</td>
<td>0 (0.00)</td>
<td>0.001</td>
</tr>
<tr>
<td>Medium</td>
<td>0 (0.00)</td>
<td>16 (64.00)</td>
<td>0.001</td>
</tr>
<tr>
<td>Strong</td>
<td>0 (0.00)</td>
<td>9 (36.00)</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Endothelium of villi</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>21 (84.00)</td>
<td>0 (0.00)</td>
<td>0.005</td>
</tr>
<tr>
<td>Faint</td>
<td>4 (16.00)</td>
<td>0 (0.00)</td>
<td>0.001</td>
</tr>
<tr>
<td>Medium</td>
<td>0 (0.00)</td>
<td>19 (76.00)</td>
<td>0.005</td>
</tr>
<tr>
<td>Strong</td>
<td>0 (0.00)</td>
<td>6 (24.00)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

(2-tailed) independent t-test; significant at p≤0.05; not significant at p>0.05.
**Figure (1):** Section of placenta showing A- Control group, immunohistochemical of NLRP3 showing negative in trophoblast (T), stroma (S) and endothelium (E). IHC, 40x, scale bar 50 µm. B- Control group, immunohistochemical of NLRP3 showing faint in trophoblast (T), stroma (S) and endothelium (E). IHC, 40x, scale bar 50 µm

**Figure (2):** Section of placenta showing A- Gestational hypertension group, immunohistochemical of NLRP3 showing medium in trophoblast (T), stroma (S) and endothelium (E). IHC, 40x, scale bar 50 µm. B- Gestational hypertension group, immunohistochemical of NLRP3 showing strong in trophoblast (T), stroma (S) and endothelium (E). IHC, 40x, scale bar 50 µm
expression of NLRP3 in trophoblast cell as shown in (Figure 2). While, in control group, 20(80.00%) cases were having negative expression of NLRP3 and 5(20.00%) cases registered the faint expression of NLRP3 in trophoblast cell as shown in (Figure 1). As well as, in gestational hypertension group, 9(36.00%) cases were having strong expression of NLRP3 and 16(64.00%) cases recorded the medium expression of NLRP3 in stroma cell as shown in (Figure 2). While in control group, 20(80.00%) cases were having negative expression of NLRP3 and 5(20.00%) cases registered the faint expression of NLRP3 in stroma cell as shown in (Figure 1). In addition to, in gestational hypertension group, 6(24.00%) cases recorded strong expression of NLRP3 and 19(76.00%) cases were having medium expression of NLRP3 as shown in (Figure 2). Although, in control group, 21(84.00%) cases were having negative expression of NLRP3 and 4(16.00%) cases displayed the faint expression of NLRP3 as shown in (Figure 1).

4. Discussion
In the current study, NLRP3 localization and intensity was increased in trophoblastic cell, stroma and endothelium of villi of gestational hypertension group (GH) compare with normal women. Increased expression of NLRP3, caspase-1 and IL-1β in the cytotrophoblast, syncytiotrophblast, fetal endothelium and stroma leucocytes in hypertensive placenta compare with control and stated distinct staining of NLRP3 was observed towards the apical side of the syncytiotrophoblast layer (Stødle, et al. [7]). According to (Pontillo, et al. [8], Tamura, et al. [9]) showing the NLRP3 are expressed in placental lysates and primary trophoblasts. (Weel, et al. [10]) also suggest that this anti-angioenic factor and other inflammatory molecules, for instance inflammatory cytokins, syntheized via syncytiotrophoblast may induce higher inflammasome expression in the placental tissue. Likewise (Hung, et al. [11], Raghupathy, et al. [12]) suggested that ischemia and hypoxia resulting from the unsuitable trophoblast invasion, result in more expression and increased production of
proinflammatory cytokines in placentas from pregnant women with preeclampsia, cytokines are formed by macrophages, Hofbour cell, within the placenta, but in addition by trophoblast.

5. Conclusions
In conclusion, the node like receptor pyrin 3 stain was much stronger in the gestational hypertension group compare with control. It mainly concentrated at the trophoblastic cell, stroma and endothelium of villi, this is an indication that NLRP3 protein is high in hypertensive pregnant women's placenta compare with normotensive placenta.

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Author Contribution
Najem, A, performed the study, examined and reviewed results, and manuscript writing with the help and supervision of Abdulhameed, WA, and AL-Bakri, N.

Conflict of Interest
The authors declare no conflict of interest.

Ethical Clearance
The study was approved by the Ethical Approval Committee.

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