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Systematic Review

Mechanism behind the Upregulation of Proteins Associated with the NLRP3 Inflammasome in Periodontitis and Their Role in the Immune Response in Diabetes—A Systematic Review

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Abstract: Background: The molecular crosstalk between periodontitis and diabetes is well established. The role of the NLRP3 inflammasome, a multicomponent inflammatory machinery, is an emerging field of research on the relationship between these two uncommunicable diseases. Recent advances are revealing further molecular details regarding the biological function and the mechanism behind the NLRP3 inflammasome dysregulation and highlighting an unexpected role for the caspase-1 in immune homeostasis. We aimed to understand which metabolic checkpoints are involved in contributing to and instigating the relationship between periodontitis and diabetes. We tried to explore the involvement of the NLRP3 in regulating the cytokine-chemokines profile and discussed the potential synergism in these mechanisms when these two diseases coexist in the same patient. Methods: A literature search was carried out in the electronic databases (MEDLINE, EMBASE, and Cochrane Library) for relevant studies from inception until January 2022 for trials and cohort studies that investigated the activation and regulation mechanism of the NLRP3 inflammasome in patients with periodontitis and type two diabetes. Two investigators independently extracted data. The data quality assessment was rated by the Joanna Briggs Institute (JBI). Results: from twenty-six references identified, three studies (two case-control and one cross-sectional) met the inclusion criteria. Analysis of periodontal tissue samples in diabetic individuals exhibited significant overexpression of the NLRP3 inflammasome when compared with healthy controls. Conclusions: there is insufficient evidence to sustain the involvement of the upregulation of genes and proteins involved in the activation of NLRP3 inflammasome components in patients with periodontitis and diabetes.

Keywords: periodontitis; diabetes; NLRP3 inflammasome; immune response

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1. Introduction

Periodontitis is one of the main causes of tooth loss in adulthood [1]. It is an infectious-inflammatory process defined by a complex of architectural and pathophysiological changes, leading to the alteration of functional and structural properties at the tissue level [2]. The development of periodontitis is caused by a confluence of elements, some of which are inherited (susceptibility) and local (bacterial plaque). This causes pathogenic crosstalk

between the innate immune system and the adaptive immune system [3]. It is characterized by acute or chronic immunological activation in response to periodontopathogen stimuli, creating a positive feedback loop with local production of the downstream pro-inflammatory molecules of the inflammasome, which are responsible for the destruction of tissue in the periodontium [4,5]. Type two diabetes is a polygenic disease determined by chronic disturbance of glucose metabolism [6]. It is characterized by relative insulin deficiency due to pancreatic β-cell dysfunction and insulin resistance [7]. Induction of insulin resistance is linked to a state of chronic inflammation, which may be identified by an increase in the secretion of inflammatory cytokines, such as interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α), and resistin, as well as a reduction in the production of the anti-inflammatory adipokine adiponectin [8]. Within this context, the NLRP3 inflammasome has been shown to have a role in the etiology of diabetes by acting as a sensor for danger signals and for high deposition of amyloid polypeptides in pancreatic islets and contributing to insulin resistance [9]. It is a consistent finding that periodontal infection acts in an additive manner to decrease diabetes control and that patients with diabetes exhibit an increase in the prevalence of periodontitis compared with subjects with normal glucose tolerance [7,10]. A mechanistically plausible model tends toward a rational model concerning the inflammatory mechanism in virtue of prolonged cytokine and chemokine secretion as a consequence of chronic exposure to inflammatory insults [11–15]. There exists overwhelming evidence that the associative evidence on type two diabetes and periodontitis is the consequence of the inflammatory vicious circle, which leads to the stimulation of a pro-inflammatory state and the subsequent chronic low-grade inflammation leading to the production of a pro-inflammatory state and, as a result, persistent low-grade inflammation [6]. The inflammation secondary to a prolonged illness state disrupts the immune balance, and the progression process of both diseases is constant [7]. The comorbidity of diabetes and periodontal disease can present as independent and parallel outcomes with no apparent direct causal connection [8,9]. It is reasonable to assume that periodontal risk will be associated with poorer glycemic control (increasing HbA1c), whereas the presence of a comorbid periodontal disorder could interfere with the management of diabetes by influencing impaired glucose tolerance and insulin resistance [10,11]. Developing data on the association between diabetes and periodontitis have highlighted the potential of the NLRP3 inflammasome as a pivotal component of the immune reaction [12]. NLRP3 is an intracellular immune receptor that is directly associated with lipid metabolism disorders and the inflammatory response, and the activation of NLRP3 plays an important role in the functioning of the innate immune system [16,17]. The NLRP3 inflammasome is a tripartite protein composed of a C-terminal leucine-rich repeat (LRR) domain, an amino-terminal pyrin domain (PYD), a central nucleotide-binding and oligomerization domain (NOD; also known as the NACHT domain), and the pro-caspase-1 enzyme. PYD and caspase-recruitment domains are both included in apoptosis-associated speck-like proteins containing a CARD (ASC). CARD and these catalytic domains, also known as p20 and p10 subunits, are the components that make up pro-caspase-1 [18]. At the protein level, NLRP3 expression is specifically upregulated in diabetes, playing a crucial role in obesity-induced inflammation as well as the in genesis, progression, and development of diabetes and its consequences. This upregulation occurs at the protein level [19]. It has been demonstrated through research that animals lacking NLRP3 and ASC are prone to obesity, and as a consequence, the inflammasome pathway is inhibited [20]. The deletion of NLRP3 causes a decrease in the amount of mature IL-1 and IL-18 that is expressed in the adipose tissue of obese mice, which also results in a decrease in the number of effector T cells [21]. Of note, the positive effects of deletion of NLRP3 inflammation have been demonstrated by an improvement in insulin signaling in adipose tissues, the liver, and skeletal muscles in NLRP3−/− mice compared with control mice [22]. Recent research on models of NLRP3 knockout mice (NLRP3KO) revealed that the inflammasome signaling pathway is intimately associated with the etiology of periodontitis, showing a lower expression of mature IL-1β [23]. Consistent with this, it has been shown that the upregulation of NLRP3

expression in connective tissue increases the local release of IL-1β and that neutrophils are rapidly mobilized during the inflammatory response, showing a ubiquitous repartition between the periodontal site and the alveolar bone crest [24]. Moreover, in vitro, NLRP3 knockdown or inhibition protected mice from alveolar bone loss. The oral injection of *Porphyromonas gingivalis*, one of the major etiological agents of periodontal disease, in wild-type mice favors the increase of alveolar bone loss, gingival gene expression of pro-IL-1β and pro-IL-18, and higher NLRP3 activity compared to NLRP3-deficient mice; it ramps up early NLRP3 expression, allowing for a robust response to the injury. These findings support the hypothesis that the NLRP3 inflammasome and its downstream molecules, IL-1β and IL-18, play a role in the bone metabolism and resorption that are caused by an infection with *Porphyromonas gingivalis* [25]. It is believed that a few different variables are involved in the activation of inflammasomes when diabetes is present [15]. The purpose of this review was to re-examine the relationship between diabetes and periodontitis, in which research is provides significant insights into the role of the NLRP3 inflammasome. The implications of such changes, their potential to influence downstream health, and the trajectory of the relationship between periodontitis and diabetes are just beginning to be realized. Here, we focused on the regulation of inflammation and placed emphasis on NLRP3-inflammasome-dependent mechanisms that facilitate intercellular communication between periodontitis and diabetes.

2. Materials and Methods

2.1. Review Design

A systematic review was carried out in compliance with the recommendations provided by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [26]. The databases of MEDLINE and Embase as well as the databanks of the Cochrane library were searched to locate relevant trials that were published between 2015 and 2022. The potentially relevant papers were searched for references, and those references were examined for further records. We also scrutinized the reference lists of the selected articles, as well as review articles on the topic, and screened for grey literature. The studies were chosen by two reviewers who were blinded (EF and BR). Disagreements were resolved by reaching a unanimous decision. The PICOT (which stands for Population, Intervention, Comparison(s), Outcome, and Time/Study Period and Date Started) strategy was utilized in order to generate the guiding question that was to be used for the execution of the study. In order to pick the MeSH terms, the search strategy was executed by utilizing the platforms provided by the Cochrane Library, EMBASE, and MEDLINE; we used combinations of the following MeSH terms and supplemented with a keywords or title search (Table 1): "NLRP3"[All Fields] AND ("inflammasomes"[MeSH Terms] OR "inflammasomes"[All Fields] OR "inflammasome"[All Fields]) AND ("periodontal"[All Fields] OR "periodontally"[All Fields] OR "periodontically"[All Fields] OR "periodontics"[MeSH Terms] OR "periodontics"[All Fields] OR "periodontic"[All Fields] OR "periodontitis"[MeSH Terms] OR "periodontitis"[All Fields] OR "periodontitides"[All Fields]) AND ("diabetes"[All Fields] OR "diabetes mellitus"[MeSH Terms] OR ("diabetes"[All Fields] AND "mellitus"[All Fields]) OR "diabetes mellitus"[All Fields] OR "diabetes"[All Fields] OR ("diabetes"[All Fields] OR "diabetic"[All Fields] OR "diabetics"[All Fields] OR "diabetes"[All Fields])). In order to map the number of identified entries that were included and excluded in order for the studies to be eligible for consideration, a PRISMA flowchart (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) was utilized (http://prisma-statement.org/prismastatement/flowdiagram.aspx, accessed on 12 July 2022). This flowchart was used to describe the information that was gathered during the various phases of the review (Figure 1).

Table 1. Quality assessment using the Joanna Briggs Institute (JBI) Critical Appraisal Checklist for Cohort Studies.

JBI Checklist No. Study	1. Were the Two Groups Similar and Recruited from the Same Population?	2. Were the Exposures Measured Similarly to Assign People to Both Exposed and Unexposed Groups?	3. Was the Exposure Measured in a Valid and Reliable Way?	4. Were Confounding Factors Identified?	5. Were Strategies to Deal with Confounding Factors Stated?	6. Were the Groups/ Participants Free of the Outcome at the Start of the Study (or at the Moment of Exposure)?	7. Were the Outcomes Measured in a Valid and Reliable Way?	8. Was the Follow-Up Time Reported and Sufficient to Be Long Enough for Outcomes to Occur?	9. Was Follow-Up Complete, and If Not, Were the Reasons for Loss of Follow-Up Described and Explored?	10. Were Strategies to Address Incomplete Follow-Up Utilized?	11. Was Appropriate Statistical Analysis Used?
Huang X. et al., 2015 [27]	Y	Y	Y	N	N	Y	Y	N	Y	Y	Y
Yi X. et al., 2019 [28]	Y	Y	Y	Y	N	N	N	Y	Y	Y	Y
García-Hernández AL. et al., 2019 [29]	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y

Exclusion based on ≥3 criterion not met; N: No; and Y: Yes.

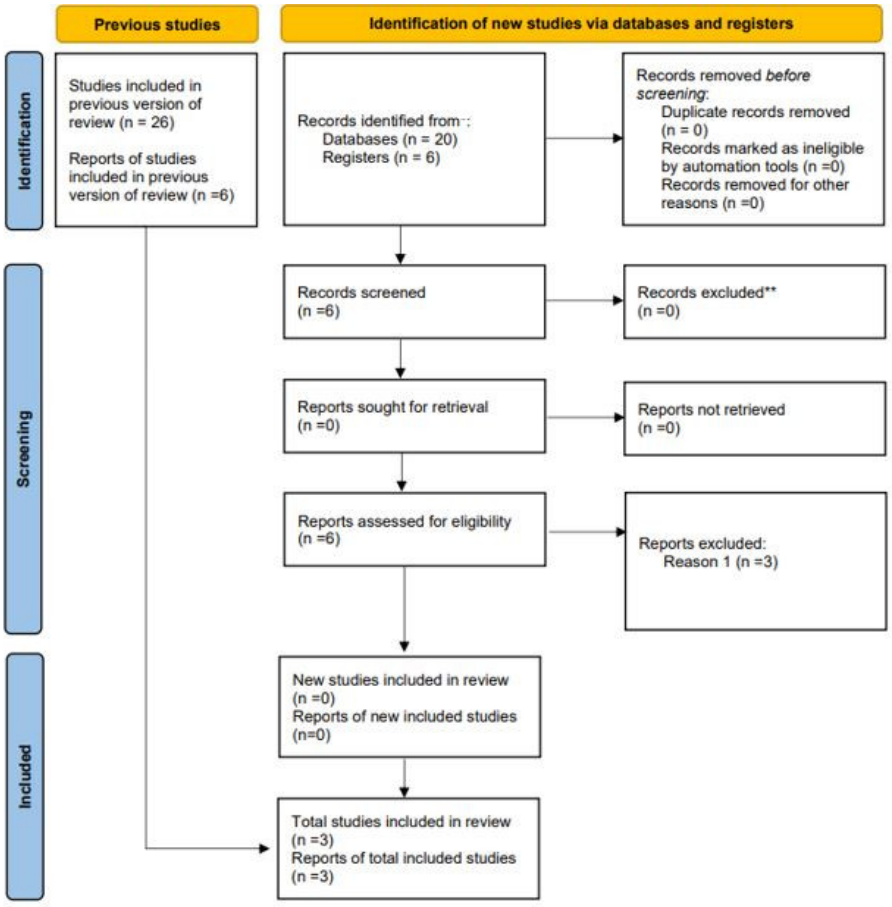

Figure 1. Study selection process.

2.2. Data Extraction and Selection

In accordance with the criteria for inclusion and exclusion, the procedure for locating, screening, and incorporating those studies was as shown in the PRISMA flow chart. After screening, the studies were identified, selected, and evaluated to determine whether or not they were eligible and whether or not they adhered to the PRISMA 2020 criteria. A schematic of the search is depicted in Figure 1. All titles and abstracts were independently

screened by two authors (EF and BR) to determine pertinent studies and to exclude studies violating the inclusion criteria. The selection of primary studies was governed by the following below mentioned eligibility criteria. An electronic data abstraction form was used to systemically collect data from the trials in the development of the report. No language restrictions were applied. To ensure literature saturation, through manual research, we conducted a comprehensive examination of additional studies that had the potential to be eligible. These studies included those that were included in previously established reviews as well as reference lists of retrieved articles. Disagreements between the reviewers were reconciled via arbitration by a third reviewer. In the event that there was a disagreement that could not be resolved, the decision was supposed to be made by a third reviewer who was an expert in the field; however, there were no conflicts that could not be resolved; therefore, this step was not required. We decided to undertake data extraction from the articles and record the results in a spreadsheet. The spreadsheet included columns for the type of study, number of participants, comparator (if any), primary outcome, type of study, and level of evidence. A narrative synthesis of studies that satisfied the inclusion criteria was carried out so that the review would be structured appropriately. In the interest of openness and exhaustiveness, a checklist was utilized for the review.

2.3. Eligibility Criteria

Articles that were published between the years 2000 and 2022 were taken into consideration for the study. We considered the studies that included animal or human subjects. Eligibility criteria comprised population-based randomized controlled trials, observational studies, and animal studies assessing the functional link between NLRP3 inflammasome, periodontitis, and the pathogenesis of diabetes. The selection process involved excluding certain types of literature such as review papers, qualitative studies, case reports, opinion pieces or comments, letters or editorials, conference abstracts, posters, and book chapters.

2.4. Quality Assessment

As shown in Table 1, data quality assessment was rated using the Joanna Briggs Institute (JBI) Critical Appraisal Checklist for Cohort Studies [30]. The tool included an evaluation of various domains that may impact the level of certainty, such as limitations in study design and execution, inconsistencies or heterogeneity, reliability of methods employed for the measurements of outcomes, imprecision, reverse causality, and publication bias. Additionally, it also considers domains that can enhance the level of certainty, such as a large magnitude effect, opposing plausible residual bias or confounding, and the appropriateness of statistical analysis. The discrepancies were addressed through a productive discussion involving a third author.

3. Results

A total of 26 studies were positioned as a result of the preliminary search. Of these, following screening and application of the inclusion and exclusion criteria, only three clinical studies remained after all the filters and crosswords were applied, and these were the ones that made up the final sample (Tables 2 and 3). Figure 1 displays the flowchart (PRISMA) of the bibliographic searches and demonstrates the results obtained initially and after applying the filters "in vivo," "clinical study," and "period from 2000 to 2022," indicating the scarcity of clinical trials on the topic. After applying filters based on sources of data collection, a total of three papers emerged as relevant for this section and were considered for analysis. The selection process is outlined in Figure 1. The three studies that fulfilled the eligibility criteria (Table 3) were observational (one cross-sectional and two case-control studies) and examined the role of the NRLP3 in mediating the relationship between periodontitis and diabetes. Unfortunately, these studies presented their results in various formats, making meta-analysis impossible.

Table 2. Summary of all papers included.

Author	Year	Study Design	Population	Samples	Experimental Analysis	Main Findings
Huang X. et al. [27]	2015	Case-control	20 healthy individuals; 20 patients diagnosed with periodontitis; 12 subjects presenting periodontitis and type 2 diabetes.	Human gingival epithelial cells (HGECs).	Immunohistochemistry (IHC).	Expression levels of NLRP3, apoptosis-associated speck-like protein containing a CARD **(ASC)**, caspase-1, and IL-1β proteins were elevated in the cytoplasm of HGECs in the context of CP and/or T2DM ($p = 0.00$) in comparison with healthy controls. Additionally, IL-1β secretion and expression of caspase-1 were decreased after the inhibition of NLRP3.
Yi X. et al. [28]	2019	Case-control	N.A.	Human periodontal ligament cells.	Quantitative real-time polymerase chain reaction (qRT-PCR); Enzyme-linked immunosorbent assay (ELISA); Western blot (WB).	A significant elevation in the expression of inflammatory cytokines (IL-6, IL-1β) was observed after AGEs stimulation for 24 h in HPDLCs ($p < 0.05$). Expression of NLRP3, a key component of the NLRP3 inflammasome and ASC, and NLRP1 was prominent after 6, 24, and 48 h of stimulation, respectively. The expression of NOD2 was unchanged in response to **advanced glycation end products** (AGEs) stimulation.
García-Hernández AL. et al. [29]	2019	Cross-sectional	18 systemically and orally healthy indivisuals; 22 patients with periodontitis with type 2 diabetes; 20 periodontal patients without diabetes.	Gingival tissue samples: the pocket epithelium (PE), the connective tissue (CT) containing inflammatory cells infiltrates (ICT), and epithelial sites adjacent to inflammatory cell infiltrates; gingival crevicular fluid.	Immunohistochemical analysis to compare NLRP3 and ASC protein expressions; RT-PCR to determine gene-expression of caspase-1, NLRP3, and ASC; ELISA to determine the levels of IL-1β and IL-18 in gingival crevicular fluid.	Samples derived from patients with periodontitis and diabetes revealed overexpression of the NLRP3 inflammasome complex when compared with the healthy group. Increased expression of CASP1 mRNA were found in periodontal group with diabetes ($p = 0.0026$). No statistically significant difference between the mRNA expressions of NLRP3 and ASC in the groups with periodontitis and or not diabetes ($p = 0.9487$; $p = 0.5042$, respectively). A higher secretion of IL-1βwas detected in patients with periodontitis and diabetes in comparison than periodontal patients ($p = 0.0018$). No statistically significant differences were registered for IL-18 production among groups.

Table 3. Excluded studies with rationale.

Number	Reference:	Summary Comment for Exclusion
1	Isola G. et al., 2021 [31]	Study not relevant to the research question and outcomes
2	Koca-Ünsal R.B. et al., 2022 [32]	Study not relevant to the research question and outcomes
3	Bagherniya M. et al., 2021 [33]	Study not relevant to the research question and outcomes
4	Lu W.L. et al., 2016 [34]	Study not relevant to the research question and outcomes
5	Zhou X. et al., 2020 [35]	Study not relevant to the research question and outcomes
6	Zahid A. et al., 2019 [36]	Study not relevant to the research question and outcomes
7	Yamaguchi Y. et al., 2021 [37]	Study not relevant to the research question and outcomes
8	Yi X. et al., 2023 [38]	Study not relevant to the research question and outcomes
9	Zhu M. et al., 2022 [39]	Study not relevant to the research question and outcomes
10	Paul O. et al., 2021 [40]	Study not relevant to the research question and outcomes
11	Chen S. et al., 2022 [41]	Study not relevant to the research question and outcomes
12	Yamaguchi Y. et al., 2017 [42]	Study not relevant to the research question and outcomes
13	Kuo H.C. et al., 2016 [43]	Study not relevant to the research question and outcomes
14	Menini S. et al., 2020 [44]	Study not relevant to the research question and outcomes
15	Lee S.I. et al., 2015 [45]	Study not relevant to the research question and outcomes
16	Faustin B. et al., 2007 [46]	Study not relevant to the research question and outcomes
17	Coto E. et al., 2018 [47]	Study not relevant to the research question and outcomes
18	Sepehri Z. et al., 2017 [48]	Study not relevant to the research question and outcomes
20	Reubold T.F. et al., 2014 [49]	Study not relevant to the research question and outcomes
21	Song Y. et al., 2017 [50]	Study not relevant to the research question and outcomes
22	Tang L. et al., 2011 [51]	Study not relevant to the research question and outcomes
23	Klen J. et al., 2015 [52]	Study not relevant to the research question and outcomes

Figure 2 shows a schematic illustration of the mechanisms of NLRP3 activation in periodontitis and diabetes.

Periodontal pathogens and hyperglicemia promote the expression of NLRP3 inflammasome leading to an inflammatory response, pyroptosis, periodontal bone resorption, and development of diabetes.

The number of participants/samples in each study ranged from 40 to 60, and a total of 32 patients with periodontitis and diabetes completed the studies until the end. The number was not specified in one study. In two of the three studies, immunochemical analysis was used, while PCR and Quantitative real-time polymerase chain reaction (qRT-PCR), Enzyme-linked immunosorbent assay (ELISA), and Western blot (WB) were used in 1 study each. As shown in Table 3: Huang X. et al. [27] revealed that NLRP3 inflammasome was upregulated in patients with periodontitis and diabetes and significantly increased IL-1β levels; Yi X. et al. [28] showed that NLRP3 mRNA as well as its protein was overexpressed in patients manifesting both diseases in comparison with the healthy group; the findings of Hernandez et al. [28] suggested that enhanced expression and activation of proteins related to the NLRP3 inflammasome may promote the impairment of periodontal inflammation and pathogenesis manifested in T2D because the protein levels of NLRP3, ASC, caspase-1, and IL -1 were higher in patients with periodontitis and diabetes.

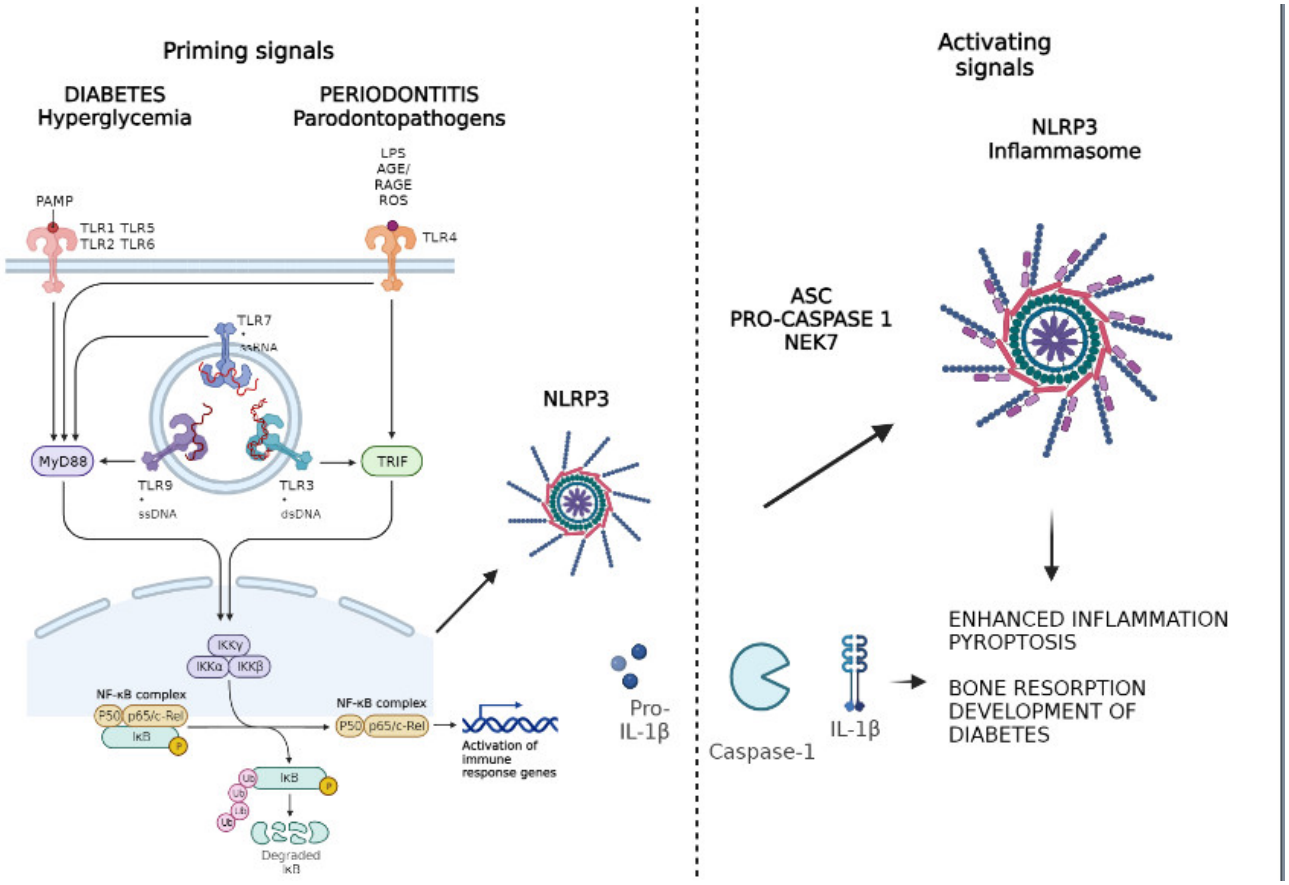

Figure 2. Schematic illustration of the mechanisms of NLRP3 activation in periodontitis and diabetes. Created with BioRender.com, accessed on 12 July 2023.

Study Bias Risk

The studies were clearly observational. The results were presented differently in the studies. Two studies clearly and objectively outlined the selection, measurement, and description of the results, but et Yi X. et al. [28] failed to describe how they carried out the measurement of the results as well as the selection of results in relation to the effect measures.

4. Discussion

Diabetes and periodontitis manifest a strong inflammatory phenotype with considerable involvement of the innate immune system, which plays a crucial role in these complex etiologies [19]. While it is generally accepted that the association between the two diseases is probabilistic (not all diabetics develop periodontitis and vice versa), the complex nature of the mechanism underlying the correlation, assuming independence, makes it difficult to estimate the exact effect size [19]. Despite the recognition of the relevance of inflammatory pathways in regulating this relationship, little is currently known about the molecular apparatus that controls these pathways. The exponential growth in our understanding of the biology of inflammasomes has led to the recognition of the NLRP3 as a common nexus among several diseases. One of the most emerging hypotheses concerning the origin of the relationship is that the pyrin domain-containing protein 3 (NLRP3) inflammasome signaling pathway mediates the correlation [17]. Given the potential in targeting the pyrin domain (PYD) in the NLRP3 for therapeutic benefit to several disorders belonging to the family of inflammatory diseases, e.g., atherosclerosis, obesity, or type two diabetes mellitus (T2DM), detecting the mechanism by which the NLRP3 is triggered in periodontitis and diabetes is attracting an increasing amount of attention. [19]. Based on these findings, our

goal was to investigate novel data on the comprehension of the NLRP3 inflammasome as well as the mechanistic knowledge of the correlation between these disorders. Both human and animal models have been utilized in the research that has been conducted on the activation of the NLRP3 inflammasome in diabetic and periodontitis patients. To date, only three studies have determined the role of the NLRP3 in modulating the relationship between diabetes and periodontitis using immunohistochemical analysis. The first evidence implicating the NLRP3 in the regulation of the interrelationships between periodontitis and diabetes arose from the study of Huang X. et al., which directly revealed the importance of the NLRP3 in the context of both diseases [27]. As a model, they employed human gingival epithelial cells (HGECs), which were generated from patients with periodontal disease who either had or did not have type two diabetes. The levels of expression of the NLRP3 inflammasome, ASC, caspase-1, and IL-1 in the samples of patients with periodontitis and/or diabetes were considerably elevated when compared with the levels of expression in healthy controls. The levels of the NLRP3 were found to be higher in diabetic individuals, and this elevation was found to have a positive correlation with the severity of the condition. Results from Hernandez and colleagues [29] confirmed this hypothesis because subjects with periodontitis and diabetes showed higher expression of NLRP3, CASP1, and ASC at the mRNA and protein levels in the epithelium and connective tissue in comparison to non-diabetic controls, supporting evidence of increased activation of inflammasome protein complexes, as shown by the over production of IL-1β ($p = 0.0018$) in the gingival crevicular fluid of the diabetic group affected. Moreover, IL-18 ($p = 0.0063$) was highly expressed in patients with periodontitis or diabetes compared with healthy subjects. Microarray analyses in experimental diabetes have added new information. Consistent with their previous study, Yi X. et al. [28] showed that the expression of the NLRP3 inflammasome was increased in gingival tissues, along with ASC, caspase-1, and pro IL-1β. These findings were observed in a mouse model of diabetes and co-occurring periodontal infections. In this randomized study, it was demonstrated that metformin could contrast NLRP3-relevant inflammatory reactions with peripheral and systemic benefits, as revealed by partial reversion of NLRP3 expression in gingival tissue and impairment of fasting glucose. Moreover, the authors examined how the expression of NLRPs changed with and without the inhibition of receptor for AGEs (RAGE) or the NF-κB pathway under the influence of AGEs. To address the effect of AGEs on NLRP3 inflammasome activation in the periodontium, exogenous AGEs were injected into human periodontal ligament cells. When compared with the control samples, Western blot analysis revealed that the NLRP3 inflammasome was significantly attenuated. RAGE and nuclear factor κB (NF-κB) pathway knockdown and inhibition is an important example of the impact of removing a critical central cellular pro-inflammatory signal pathway. Overall, there are close connections between the repertoire of proteins and pro-inflammatory cytokines that have a great impact on establishing the relationship between diabetes and periodontitis. Thus, the evidence to date is incomplete pertaining to the involvement and possible underlying molecular mechanism. Moreover, unexplored factors may alter the findings. Overall, these data suggest a possible bidirectional feedback regulatory mechanism between periodontitis, diabetes, and NLRP3-related inflammation. The cellular metabolic profile which induces the reciprocal changes in both diseases requires further analysis.

5. Conclusions

According to the findings of a number of studies, the NLRP3 inflammasome may play an important role as a fundamental signaling structure in the connection between diabetes and periodontitis. This would make it an important link between the two diseases. However, our knowledge is not sufficiently complete to infer the exact mechanism that involves a coherent chain linking periodontitis and diabetes through the NLP3 inflammasome, and there is insufficient evidence from independently funded clinical studies to support or refute the implication of the NLRP3 inflammasome. It will be necessary to conduct longitudinal research before these findings can be applied in a therapeutic setting.

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References

1. Könönen, E.; Gursoy, M.; Gursoy, U.K. Periodontitis: A Multifaceted Disease of Tooth-Supporting Tissues. *J. Clin. Med.* **2019**, *8*, 1135. [CrossRef] [PubMed]
2. Loos, B.G.; Papantonopoulos, G.; Jepsen, S.; Laine, M.L. What is the Contribution of Genetics to Periodontal Risk? *Dent. Clin. N. Am.* **2015**, *59*, 761–780. [CrossRef] [PubMed]
3. Hajishengallis, G.; Chavakis, T.; Lambris, J.D. Current understanding of periodontal disease pathogenesis and targets for host-modulation therapy. *Periodontol. 2000* **2020**, *84*, 14–34. [CrossRef] [PubMed]
4. Schätzle, M.; Löe, H.; Lang, N.P.; Heitz-Mayfield, L.J.; Bürgin, W.; Anerud, A.; Boysen, H. Clinical course of chronic periodontitis. III. Patterns, variations and risks of attachment loss. *J. Clin. Periodontol.* **2003**, *30*, 909–918. [CrossRef]
5. Lamont, R.J.; Koo, H.; Hajishengallis, G. The oral microbiota: Dynamic communities and host interactions. *Nat. Rev. Microbiol.* **2018**, *16*, 745–759. [CrossRef]
6. Valaiyapathi, B.; Gower, B.; Ashraf, A.P. Pathophysiology of Type 2 Diabetes in Children and Adolescents. *Curr. Diabetes Rev.* **2020**, *16*, 220–229. [CrossRef]
7. Lyssenko, V.; Jonsson, A.; Almgren, P.; Pulizzi, N.; Isomaa, B.; Tuomi, T.; Berglund, G.; Altshuler, D.; Nilsson, P.; Groop, L. Clinical risk factors, DNA variants, and the development of type 2 diabetes. *N. Engl. J. Med.* **2008**, *359*, 2220–2232. [CrossRef]
8. Rapone, B.; Ferrara, E.; Santacroce, L.; Topi, S.; Converti, I.; Gnoni, A.; Scarano, A.; Scacco, S. Gingival Crevicular Blood as a Potential Screening Tool: A Cross Sectional Comparative Study. *Int. J. Environ. Res. Public. Health* **2020**, *17*, 7356. [CrossRef]
9. Ward, R.; Li, W.; Abdul, Y.; Jackson, L.; Dong, G.; Jamil, S.; Filosa, J.; Fagan, S.C.; Ergul, A. NLRP3 inflammasome inhibition with MCC950 improves diabetes-mediated cognitive impairment and vasoneuronal remodeling after ischemia. *Pharmacol. Res.* **2019**, *142*, 237–250. [CrossRef]
10. Scaramuzza, A.; Cherubini, V.; Tumini, S.; Bonfanti, R.; Buono, P.; Cardella, F.; d'Annunzio, G.; Frongia, A.P.; Lombardo, F.; Monciotti, A.C.; et al. Diabetes Study Group of the Italian Society for Pediatric Endocrinology and Diabetology. Recommendations for self-monitoring in pediatric diabetes: A consensus statement by the ISPED. *Acta Diabetol.* **2014**, *51*, 173–184. [CrossRef]
11. Wu, C.Z.; Yuan, Y.H.; Liu, H.H.; Li, S.S.; Zhang, B.W.; Chen, W.; An, Z.J.; Chen, S.Y.; Wu, Y.Z.; Han, B.; et al. Epidemiologic relationship between periodontitis and type 2 diabetes mellitus. *BMC Oral. Health* **2020**, *20*, 204. [CrossRef]
12. Nascimento, G.G.; Leite, F.R.M.; Vestergaard, P.; Scheutz, F.; López, R. Does diabetes increase the risk of periodontitis? A systematic review and meta-regression analysis of longitudinal prospective studies. *Acta Diabetol.* **2018**, *55*, 653–667. [CrossRef]
13. Poudel, P.; Griffiths, R.; Wong, V.W.; Arora, A.; Flack, J.R.; Khoo, C.L.; George, A. Oral health knowledge, attitudes and care practices of people with diabetes: A systematic review. *BMC Public Health* **2018**, *18*, 577. [CrossRef]
14. Stöhr, J.; Barbaresko, J.; Neuenschwander, M.; Schlesinger, S. Bidirectional association between periodontal disease and diabetes mellitus: A systematic review and meta-analysis of cohort studies. *Sci. Rep.* **2021**, *11*, 13686. [CrossRef]
15. Botero, J.E.; Rodríguez, C.; Agudelo-Suarez, A.A. Periodontal treatment and glycaemic control in patients with diabetes and periodontitis: An umbrella review. *Aust. Dent. J.* **2016**, *61*, 134–148. [CrossRef]
16. Swanson, K.V.; Deng, M.; Ting, J.P.Y. The NLRP3 inflammasome: Molecular activation and regulation to therapeutics. *Nat. Rev. Immunol.* **2019**, *19*, 477–489. [CrossRef]
17. Cocco, M.; Pellegrini, C.; Martínez-Banaclocha, H.; Giorgis, M.; Marini, E.; Costale, A.; Miglio, G.; Fornai, M.; Antonioli, L.; López-Castejón, G.; et al. Development of an Acrylate Derivative Targeting the NLRP3 Inflammasome for the Treatment of Inflammatory Bowel Disease. *J. Med. Chem.* **2017**, *60*, 3656–3671. [CrossRef]
18. Mangan, M.S.J.; Olhava, E.J.; Roush, W.R.; Seidel, H.M.; Glick, G.D.; Latz, E. Targeting the NLRP3 inflammasome in inflammatory diseases. *Nat. Rev. Drug Discov.* **2018**, *17*, 588–606; Erratum in **2018**, *17*, 688. [CrossRef]
19. Aganna, E.; Martinon, F.; Hawkins, P.N.; Ross, J.B.; Swan, D.C.; Booth, D.R.; Lachmann, H.J.; Bybee, A.; Gaudet, R.; Woo, P.; et al. Association of mutations in the NALP3/CIAS1/PYPAF1 gene with a broad phenotype including recurrent fever, cold sensitivity, sensorineural deafness, and AA amyloidosis. *Arthritis Rheum.* **2002**, *46*, 2445–2452; Erratum in **2002**, *46*, 3398. [CrossRef]

20. Ding, S.; Xu, S.; Ma, Y.; Liu, G.; Jang, H.; Fang, J. Modulatory mechanisms of the NLRP3 inflammasomes in diabetes. *Biomolecules* **2019**, *9*, 850. [CrossRef]
21. Kim, Y.; Wang, W.; Okla, M.; Kang, I.; Moreau, R.; Chung, S. Suppression of NLRP3 inflammasome by γ-tocotrienol ameliorates type 2 diabetes. *J. Lipid Res.* **2016**, *57*, 66–76. [CrossRef] [PubMed]
22. Gora, I.M.; Ciechanowska, A.; Ladyzynski, P. NLRP3 Inflammasome at the Interface of Inflammation, Endothelial Dysfunction, and Type 2 Diabetes. *Cells* **2021**, *10*, 314. [CrossRef] [PubMed]
23. Kelley, N.; Jeltema, D.; Duan, Y.; He, Y. The NLRP3 Inflammasome: An Overview of Mechanisms of Activation and Regulation. *Int. J. Mol. Sci.* **2019**, *20*, 3328. [CrossRef] [PubMed]
24. Chen, Y.; Yang, Q.; Lv, C.; Chen, Y.; Zhao, W.; Li, W.; Chen, H.; Wang, H.; Sun, W.; Yuan, H. NLRP3 regulates alveolar bone loss in ligature-induced periodontitis by promoting osteoclastic differentiation. *Cell Prolif.* **2021**, *54*, e12973. [CrossRef]
25. Page, M.J.; Moher, D.; Bossuyt, P.M.; Boutron, I.; Hoffmann, T.C.; Mulrow, C.D.; Shamseer, L.; Tetzlaff, J.M.; Akl, E.A.; Brennan, S.E.; et al. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. *BMJ* **2021**, *372*, n71. [CrossRef]
26. Aromataris, E.; Fernandez, R.; Godfrey, C.; Holly, C.; Kahlil, H.; Tungpunkom, P. Summarizing systematic reviews: Methodological development, conduct and reporting of an Umbrella review approach. *Int. J. Evid. Based Healthc.* **2015**, *13*, 132–140. [CrossRef]
27. Huang, X.; Yang, X.; Ni, J.; Xie, B.; Liu, Y.; Xuan, D.; Zhang, J. Hyperglucose contributes to periodontitis: Involvement of the NLRP3 pathway by engaging the innate immunity of oral gingival epithelium. *J. Periodontol.* **2015**, *86*, 327–335. [CrossRef]
28. Yi, X.; Zhang, L.; Lu, W.; Tan, X.; Yue, J.; Wang, P.; Xu, W.; Ye, L.; Huang, D. The effect of NLRP inflammasome on the regulation of AGEs-induced inflammatory response in human periodontal ligament cells. *J. Periodontal Res.* **2019**, *54*, 681–689. [CrossRef]
29. García-Hernández, A.L.; Muñoz-Saavedra, Á.E.; González-Alva, P.; Moreno-Fierros, L.; Llamosas-Hernández, F.E.; Cifuentes-Mendiola, S.E.; Rubio-Infante, N. Upregulation of proteins of the NLRP3 inflammasome in patients with periodontitis and uncontrolled type 2 diabetes. *Oral Dis.* **2019**, *25*, 596–608. [CrossRef]
30. Zhou, X.; Zhang, P.; Wang, Q.; Ji, N.; Xia, S.; Ding, Y.; Wang, Q. Metformin ameliorates experimental diabetic periodontitis independently of mammalian target of rapamycin (mTOR) inhibition by reducing NIMA-related kinase 7 (Nek7) expression. *J. Periodontol.* **2019**, *90*, 1032–1042. [CrossRef]
31. Isola, G.; Polizzi, A.; Santonocito, S.; Alibrandi, A.; Williams, R.C. Periodontitis activates the NLRP3 inflammasome in serum and saliva. *J. Periodontol.* **2022**, *93*, 135–145. [CrossRef]
32. Koca-Ünsal, R.B.; Şehirli, A.Ö.; Sayıner, S.; Aksoy, U. Relationship of NLRP3 inflammasome with periodontal, endodontic and related systemic diseases. *Mol. Biol. Rep.* **2022**, *49*, 11123–11132. [CrossRef]
33. Bagherniya, M.; Khedmatgozar, H.; Fakheran, O.; Xu, S.; Johnston, T.P.; Sahebkar, A. Medicinal plants and bioactive natural products as inhibitors of NLRP3 inflammasome. *Phytother. Res.* **2021**, *35*, 4804–4833. [CrossRef]
34. Lu, W.L.; Song, D.Z.; Yue, J.L.; Wang, T.T.; Zhou, X.D.; Zhang, P.; Zhang, L.; Huang, D.M. NLRP3 inflammasome may regulate inflammatory response of Human Periodontal Ligament Fibroblasts in an apoptosis-associated speck-like protein containing a CARD (ASC) dependent manner. *Int. Endod. J.* **2016**, *50*, 967–975. [CrossRef]
35. Zhou, X.; Wang, Q.; Nie, L.; Zhang, P.; Zhao, P.; Yuan, Q.; Ji, N.; Ding, Y.; Wang, Q. Metformin ameliorates the NLPP3 inflammasome mediated pyroptosis by inhibiting the expression of NEK7 in diabetic periodontitis. *Arch. Oral Biol.* **2020**, *116*, 104763. [CrossRef]
36. Zahid, A.; Li, B.; Kombe, A.J.K.; Jin, T.; Tao, J. Pharmacological Inhibitors of the NLRP3 Inflammasome. *Front. Immunol.* **2019**, *5*, 2538. [CrossRef]
37. Vo, T.T.T.; Lee, C.W.; Chiang, Y.C.; Chen, Y.W.; Yu, Y.H.; Tuan, V.P.; Wu, C.Z.; Lee, I.T. Protective mechanisms of Taiwanese green propolis toward high glucose-induced inflammation via NLRP3 inflammasome signaling pathway in human gingival fibroblasts. *J. Periodontal Res.* **2021**, *56*, 804–818. [CrossRef]
38. Yi, X.; Song, Y.; Xu, J.; Wang, L.; Liu, L.; Huang, D.; Zhang, L. NLRP10 promotes AGEs-induced NLRP1 and NLRP3 inflammasome activation via ROS/MAPK/NF-κB signaling in human periodontal ligament cells. *Odontology* **2023**, 1–12. [CrossRef]
39. Zhu, M.; Zhao, Z.; Xu, H.H.K.; Dai, Z.; Yu, K.; Xiao, L.; Schneider, A.; Weir, M.D.; Oates, T.W.; Bai, Y.; et al. Effects of Metformin Delivery via Biomaterials on Bone and Dental Tissue Engineering. *Int. J. Mol. Sci.* **2022**, *23*, 15905. [CrossRef]
40. Paul, O.; Arora, P.; Mayer, M.; Chatterjee, S. Inflammation in Periodontal Disease: Possible Link to Vascular Disease. *Front. Physiol.* **2021**, *11*, 609614. [CrossRef]
41. Chen, S.; Huang, D.; Zhu, L.; Jiang, Y.; Guan, Y.; Zou, S.; Li, Y. Contribution of diabetes mellitus to periodontal inflammation during orthodontic tooth movement. *Oral Dis.* **2022**. *Epub ahead of print*. [CrossRef] [PubMed]
42. Yamaguchi, Y.; Kurita-Ochiai, T.; Kobayashi, R.; Suzuki, T.; Ando, T. Regulation of the NLRP3 inflammasome in Porphyromonas gingivalis-accelerated periodontal disease. *Inflamm. Res.* **2017**, *66*, 59–65. [CrossRef]
43. Kuo, H.C.; Chang, L.C.; Chen, T.C.; Lee, K.C.; Lee, K.F.; Chen, C.N.; Yu, H.R. Sterol Regulatory Element-Binding Protein-1c Regulates Inflammasome Activation in Gingival Fibroblasts Infected with High-Glucose-Treated Porphyromonas gingivalis. *Front. Cell. Infect. Microbiol.* **2016**, *6*, 195. [CrossRef] [PubMed]
44. Menini, S.; Iacobini, C.; Vitale, M.; Pugliese, G. The Inflammasome in Chronic Complications of Diabetes and Related Metabolic Disorders. *Cells* **2020**, *9*, 1812. [CrossRef] [PubMed]
45. Lee, S.I.; Kang, S.K.; Jung, H.J.; Chun, Y.H.; Kwon, Y.D.; Kim, E.C. Muramyl dipeptide activates human beta defensin 2 and pro-inflammatory mediators through Toll-like receptors and NLRP3 inflammasomes in human dental pulp cells. *Clin. Oral. Investig.* **2015**, *19*, 1419–1428. [CrossRef]

46. Faustin, B.; Lartigue, L.; Bruey, J.M.; Luciano, F.; Sergienko, E.; Bailly-Maitre, B.; Volkmann, N.; Hanein, D.; Rouiller, I.; Reed, J.C. Reconstituted NALP1 inflam- masome reveals two-step mechanism of caspase-1 activation. *Mol. Cell* **2007**, *25*, 713–724. [CrossRef]
47. Coto, E.; Díaz-Corte, C.; Tranche, S.; Gómez, J.; Alonso, B.; Iglesias, S.; Reguero, J.R.; López-Larrea, C.; Coto-Segura, P. Gene variants in the NF- KB pathway (NFKB1, NFKBIA, NFKBIZ) and their association with type 2 diabetes and impaired renal function. *Hum. Immunol.* **2018**, *79*, 494–498. [CrossRef]
48. Sepehri, Z.; Kiani, Z.; Afshari, M.; Kohan, F.; Dalvand, A.; Ghavami, S. Inflammasomes and type 2 diabetes: An updated systematic review. *Immunol. Lett.* **2017**, *192*, 97–103. [CrossRef]
49. Reubold, T.F.; Hahne, G.; Wohlgemuth, S.; Eschenburg, S. Crystal struc- ture of the leucine-rich repeat domain of the NOD-like receptor NLRP1: Implications for binding of muramyl dipeptide. *FEBS Lett.* **2014**, *588*, 3327–3332. [CrossRef]
50. Song, Y.; Wang, Y.; Zhang, Y.; Geng, W.; Liu, W.; Gao, Y.; Li, S.; Wang, K.; Wu, X.; Kang, L.; et al. Advanced glycation end prod- ucts regulate anabolic and catabolic activities via NLRP3-inflam- masome activation in human nucleus pulposus cells. *J. Cell. Mol. Med.* **2017**, *21*, 1373–1387. [CrossRef]
51. Tang, L.; Zhou, X.D.; Wang, Q.; Zhang, L.; Wang, Y.; Li, X.Y.; Huang, D.M. Expression of TRAF6 and pro-in- flammatory cytokines through activation of TLR2, TLR4, NOD1, and NOD2 in human periodontal ligament fibroblasts. *Arch. Oral Biol.* **2011**, *56*, 1064–1072. [CrossRef]
52. Klen, J.; Goričar, K.; Janež, A.; Dolžan, V. NLRP3 inflammasome polymorphism and macrovascular complications in type 2 diabetes patients. *J. Diabetes Res.* **2015**, *2015*, 616747. [CrossRef]