8-Oxo-7,8-Dihydro-2′-Deoxyguanosine (8-oxodG) and 8-Hydroxy-2′-Deoxyguanosine (8-OHdG) as a Potential Biomarker for Gestational Diabetes Mellitus (GDM) Development

Sandra K. Urbaniak, Karolina Boguszewska, Michał Szewczuk, Julia Kaźmierczak-Barańska and Bolesław T. Karwowski *

DNA Damage Laboratory of Food Science Department, Faculty of Pharmacy, Medical University of Lodz, ul. Muszynskiego 1, 90-151 Lodz, Poland; sandra.urbaniak@stud.umed.lodz.pl (S.K.U.); karolina.boguszewska@stud.umed.lodz.pl (K.B.); michal.szewczuk@stud.umed.lodz.pl (M.S.); julia.kazmierczak-baranska@umed.lodz.pl (J.K.-B.)

* Correspondence: boleslaw.karwowski@umed.lodz.pl; Tel.: +48-42-677-9140; Fax: +48-42-678-8398

Academic Editor: Katherine Seley-Radtke
Received: 22 November 2019; Accepted: 1 January 2020; Published: 3 January 2020

Abstract: The growing clinical and epidemiological significance of gestational diabetes mellitus results from its constantly increasing worldwide prevalence, obesity, and overall unhealthy lifestyle among women of childbearing age. Oxidative stress seems to be the most important predictor of gestational diabetes mellitus development. Disturbances in the cell caused by oxidative stress lead to different changes in biomolecules, including DNA. The nucleobase which is most susceptible to oxidative stress is guanine. Its damage results in two main modifications: 8-hydroxy-2′-deoxyguanosine or 8-oxo-7,8-dihydro-2′-deoxyguanosine. Their significant level can indicate pathological processes during pregnancy, like gestational diabetes mellitus and probably, type 2 diabetes mellitus after pregnancy. This review provides an overview of current knowledge on the use of 8-hydroxy-2′-deoxyguanosine and/or 8-oxo-7,8-dihydro-2′-deoxyguanosine as a biomarker in gestational diabetes mellitus and allows us to understand the mechanism of 8-hydroxy-2′-deoxyguanosine and/or 8-oxo-7,8-dihydro-2′-deoxyguanosine generation during this disease.

Keywords: gestational diabetes mellitus; 8-hydroxy-2′-deoxyguanosine; 8-oxo-7,8-dihydro-2′-deoxyguanosine; oxidative stress; DNA damage

1. Introduction

One of the major complications of pregnancy is gestational diabetes mellitus (GDM) [1]. According to global guidelines, GDM may be defined as being any hyperglycemic state that occurs during the second half of gestation among previously healthy pregnant women [2–4]. GDM is usually diagnosed based on positive one-step 75-g oral glucose tolerance test (OGTT), when there are no additional factors that might indicate occurrence of prior type 1 or 2 diabetes mellitus (T1DM and T2DM, respectively) [3,4].

The worldwide prevalence of GDM is estimated to range from 3% to 5% and this number is constantly growing, mainly due to increasing obesity and physical inactivity [5]. Diabetes seems to be more common in non-Caucasian populations such as: African American, Hispanic American, Native American, Pacific Islander, and South or East Asian populations [6]. Other factors include an increased body mass index (BMI), family history of diabetes, maternal age over 25, and previous GDM [7,8].

Even though GDM normally declines post-partum, a greater number of patients who are undiagnosed or diagnosed later develop T2DM following pregnancy, cardiovascular disease, or metabolic syndrome [9,10]. Hyperglycemic state also positively correlates with an increased fetal
infection rate and mortality. Offspring, like their mother, in adulthood, have a greater predisposition to develop T2DM, metabolic syndrome, and obesity [11].

Although many studies on the pathophysiology of GDM have been conducted, the precise mechanism of its development remains unclear. Hyperglycemia occurring in GDM women is probably the result of β-cells progressive dysfunction [12]. Growing evidence indicates inflammation [13,14], disruption of the insulin signaling pathway [15], plasma adipokine levels alteration [16], and endoplasmic reticulum stress [14] as some of the reasons for the above process. However, oxidative stress (OS) is considered to be the primary cause of GDM development and its components should be strictly controlled during pregnancy and treatment [17].

Purines are organic compounds which can undergo oxidation reaction and form different products [18]. Among the most important biological markers of OS the following ones belonging to purines may be distinguished: 8-hydroxy-2′-deoxyguanosine (8-OHdG) or its oxidized form—8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG). Although all living cells develop a broad range of DNA repair mechanisms, their enzymatic repair system does not always lead to a complete removal of all DNA modifications. Therefore, misrepaired DNA constitutes a major problem for cells, mainly because of genetic information changes as well as mutagenesis and cell apoptosis connected with them [19]. Growing evidence proves that accumulation of abundant lesions, mainly 8-OHdG or 8-oxodG, is an important factor indicating development of GDM, and in the aftermath, T2DM in mothers and their offspring [20,21].

This review summarizes the current knowledge about the impact of oxidative guanine (G) base damages, mainly 8-OHdG or its oxidized form 8-oxodG on GDM development. Additionally, biomarkers of GDM and several mechanisms connected with OS induction and GDM development are described.

2. The Influence of ROS Overproduction on GDM Development

Reactive oxygen species (ROS) are highly reactive derivatives of oxygen molecules arising as a result of incomplete oxygen reduction. Their high reactivity is determined by the existence of at least one unpaired electron on the valence shell [22].

About 5% of the total inhaled oxygen is converted into ROS. Those most important ones are, among others: superoxide radical (O$_2^{-}$), hydrogen peroxide (H$_2$O$_2$), hydroxyl radical (•OH), and singlet oxygen ($^1$O$_2$) [23] ROS are produced mainly in response to radiation (alfa, beta, gamma, X-radiation (X-ray)) or Ultraviolet-Visible (UV-Vis), inflammation (infections), chronic diseases (alcoholism, injuries, cancer), chemical compounds (pesticides, benzopyrene, nitrous oxide), metabolic processes (peroxidation of fatty acids), and metabolic disorders (diabetes mellitus). Moreover, ROS are derived from normal physiological processes conducted in various cellular compartments [24]. In physiological conditions, these processes maintain ROS at the right level and provide proper reduction and oxidation reactions in the respiratory chain, transport of oxygen by hemoglobin, regeneration of energy sources, phagocytosis process, gene expression regulation, and activation of cytochrome P450 [25].

However, excessive production of ROS and insufficient work of antioxidant defense mechanism can lead to a condition known as oxidative stress [25,26]. Oxidative stress is defined as an imbalance towards prooxidative changes which leads to damage and dysfunction of cellular structures, cells and whole organisms. This imbalance is associated with overproduction of free radicals in mitochondria, with formation of advanced glycation end products (AGEs) or as a result of activation of protein kinase C (PKC), polyol or hexoamine pathway in response to elevated glucose levels [27]. Increased ROS production is often accompanied by reduced activity of antioxidant systems, which involves deepening of the OS (Figure 1) [25,27].
Properly stimulate the production and activation of antioxidants and, ultimately, maintain the ROS ratio [35,36], and finally reduced antioxidant capacity of the glutathione system [36].

Sorbitol-induced osmotic stress, reduction of Na\(^+\)/K\(^+\) ATPase activity, a decrease of NADPH/NADP ratio [35,36], and finally reduced antioxidant capacity of the glutathione system [36].

Inhibition of endothelial nitric oxide synthase (eNOS) [31], activation of NADPH Oxidase 5 (NOX5) [32], stimulation of superoxide anion formation [33], and mediation of fatty acid-induced β-cell apoptosis [34].

Another activated pathway—the polyol pathway, leads to the reduction of glucose in fructose 3-phosphate (fructose-3-P) caused by sorbitol dehydrogenase. As a consequence, this leads to sorbitol-induced osmotic stress, reduction of Na\(^+\)/K\(^+\) ATPase activity, a decrease of NADPH/NADP ratio [35,36], and finally reduced antioxidant capacity of the glutathione system [36].
Activation of the hexosamine pathway, the third of the mentioned pathways, causes accumulation of nucleotide sugar—Uridine diphosphate \(N\)-acetylglucosamine (UDP-GLC-NAc) and its oxygen derivatives. UDP-GLC-NAc leads to pathological expression of transforming growth factor-beta (TGF\(\beta\)1) genes [37].

The effect of ROS on cellular processes mainly depends on the strength and duration of exposure. The destructive action of radicals can include almost all of the biomolecules which occur in the body—protein modifications, lipid peroxidation, and DNA mutations [23,25]. Ultimately, cells abandon the cell cycle and enter the G0 phase or, in the case of permanent exposure and/or a high concentration of ROS, activate the process of programmed cell death [38].

3. Types of the Antioxidant Defense Mechanisms

Organisms have created various integrated antioxidant defense mechanisms which neutralize or reduce the negative effects of ROS and lead to the “oxidative balance”. The proper concentration of antioxidants is necessary for maintaining basic cell functions (proliferation, differentiation, energy production) and a whole organ functioning at the right level and its activity can change depending on intracellular concentration of ROS. The antioxidative defense system includes enzymatic (superoxide dismutase (SOD), catalase (CAT), thioredoxin (TRX), peroxiredoxin (PRX), heme oxygenase-1, glutathione peroxidase (GPx), glutathione reductase (GRd), and glutathione S-transferase (GST)) and non-enzymatic (low molecular weight) (all-trans retinol 2 (Vitamin A), ascorbic acid (Vitamin C), \(\alpha\)-Tocopherol (Vitamin E), \(\beta\)-Carotene, uric acid, glutathione (GSH), and tripeptide (1-\(\gamma\)-glutamyl-1-cysteinyl-1-glycine)) antioxidants [39].

4. Biological Markers of Oxidative Stress in GDM Women

The degree of oxidative stress among women with GDM can be measured using different biomarkers [20,40] which are classified according to the reactions that change them [41]. Two classes of biological markers may be distinguished: ROS-modified molecules and antioxidant molecules altered by increased redox stress [41].

4.1. Influence of Oxidative Stress on Lipids

The main indicators of increased oxidative stress in diabetes, as well as in patients with GDM, are lipid peroxidation products that increase fluidity and permeability of the cell membrane [42,43]. Studies show increased levels of malonyl dialdehyde (MDA) in the placenta tissue, cord plasma, and maternal plasma among patients with GDM [44]. MDA reacts with thiobarbituric acid (TBA) which leads to the formation of thiobarbituric acid reactive substances (TBARS) [27]. Higher level of TBARS is observed in serum from diabetic mothers and their macrosomic offspring [45] as well as in cord blood of newborns [46]. Among lipid peroxidation products, an important indicator is also an increased concentration of lipid hydroperoxide (LOOH) in serum [47] and 8-iso-PGF2\(\alpha\), belonging to F2-isoprostanes [40,48]. TBARS and 8-iso-PGF2\(\alpha\) are used as an indirect biomarkers of OS [39].

4.2. Influence of Oxidative Stress on Proteins

Besides lipid peroxidation, protein oxidation is also presented under GDM conditions. OS can trigger formation of protein cross-linking, fragmentation of the peptide as well as formation of modified, denaturated, and non-functioning proteins as well as intensified proteolysis reactions [39]. Moreover, a hyperglycemic state can also lead to protein glycation [49]. Among patients with GDM, higher levels of advanced oxidation protein products (AOPP) [50,51], protein hydroperoxides (POOHs), protein carbonyls (PCO) [51], C-reactive protein (CRP) [40], and glycated hemoglobin (HbA1c) can be presented [51]. Moreover, ROS can also decrease the level of Paraoxonase 1 (PON1) [51] and, depending on the conducted clinical studies, cause an increased [51] or decreased [52] level of 3-nitrotyrosine (3-NT). Increased formation and accumulation of protein oxidation products is considered as an
important mediators of adipocyte disorders. They intensify inflammatory response and play a significant role in chronic development of diabetic complications [28].

4.3. Influence of Oxidative Stress on Enzymatic and Non-Enzymatic Antioxidants

Considering the damage caused by oxidative stress in GDM, the most frequently studied enzymes have been superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX). Current data indicate that the concentration of each of these enzymes is decreased [53].

In case of vitamins, the results are less clear. Concentration of individual vitamins are either reduced (vitamin C and vitamin E) [54], increased (vitamin C [45] and vitamin E [55]), or remains at the same level (vitamin A) [45]. Probably, these discrepancies can be a result of different diagnostic criteria and/or too small examined samples [27].

4.4. Influence of Oxidative Stress on DNA

Oxidative damages of nucleic acids are the most dangerous modifications observed among biomolecules. They can be distinguished into: oxidation of bases and/or sugar fragments, single/double strand breaks (SSB and DSB, respectively), basic/apurinic/apirimidinic (AP) sites, purine/pyrimidine/sugar-related modifications, structural mutations of nucleic bases, and DNA-protein cross-linking, deletions and/or translocations of chromosome fragments. The accumulation of such damages can lead to changes of genetic information, and consequently to mutagenesis and cells apoptosis [56].

The hydroxyl radical \( ^{\bullet} \text{OH} \) is responsible for the majority of DNA damages. It can attack the C5 carbon atom or carbon atom from the methyl group (CH3) presented in the pyrimidines, which is the C8 atom in the case of purines or the amino group of adenines (A) [57].

Of all the nucleic bases, G is the most susceptible to oxidative stress caused by ROS. The best-known damage is 8-Oxoguanine (8-oxoG). The latest data indicate that up to \( 10^3 \) 8-oxoG modifications are created daily in DNA per cell. The widespread occurrence of nucleobases damage is a convenient marker of DNA oxidative damage, repair, and cellular oxidative stress in general [56]. GC rich sequences presented at a transcription factor binding sites appear to be the most susceptible to damage. The appearance of 8-oxoG modification at these sites alter the expression of related with them genes [58].

Apart from 8-oxoG, other DNA lesions can be also observed, for instance 5-hydroxymethyl uracil (5-OHMeUra) or 8,5’-cyclo-2’-deoxyadenosine (cyclo-dA) [39]. The first arises as a result of 5-methylcytosine (5-MeCyt) oxidation and can introduce incorrect DNA methylation patterns, which as a consequence lead to gene silencing, a disturbance in chromatin organization, and an incorrect DNA repair mechanism [39,59]. Cyclo-dA appearing in the TATA sequence limits the interaction of proteins with this sequence, which also leads to inhibition of gene transcription [60].

5. Generation of the Most Abundant Lesions Observed in GDM: 8-OHdG and 8-oxodG

Modifications of 8-OHdG and 8-oxodG arise as a result of the interaction between \( ^{\bullet} \text{OH} \) or \( ^{\text{1}} \text{O}_2 \) and G of the DNA strand. Free radicals attack G or free 2’-deoxyguanosine, which consequently generates radical adducts. Electron abstraction forms 8-OHdG, which through a reaction known as keto-enol tautomerism, is transformed into the major oxidized product 8-oxodG (Figure 2) [61].

Simone et al. [62], have provided the first likely model of DNA damage formation in diabetes mellitus. According to their results, hyperglycemia can induce redox-dependent activation of serine/threonine-specific protein kinase—Akt which enhances phosphorylation of tuberin protein and as a consequence also enhances downregulation of human 8-oxoguanine-DNA glycosylase 1 (hOGG1), enzyme engaged in the DNA base excision repair pathway (BER).
6. Repair Mechanism of 8-OHdG and 8-oxodG in Diabetes Mellitus

Both 8-OHdG and 8-oxodG are recognized by cells as heavy lesions which need to be removed quickly. The foregoing modifications can induce transversion mutations: GC→AT, which can lead to cancer [19] or trigger high levels of oxidative stress [20]. The BER repair system, which was invented by Tomas Lindahl [63], and two executive enzymes: hOGG1 [56] and human MutT homologue (hMTH1), are responsible for removing these alterations [64].

hOGG1 is a bifunctional DNA glycosylase with lyase activity which is responsible for recognizing and the excising of 8-oxodG from the oxidatively-damaged DNA. The general repair mechanism initiated by hOGG1 includes lesion identification, base excision, phosphodiester bond 3’ cleaving, and site-specific changes in the double-helix structure of DNA [56].

hMTH1, also known as 2-Hydroxy-dATP diphosphatase or nudix hydrolase 1 (NUDT1), is an enzyme primarily localized in the cell cytoplasm that performs two functions during the DNA repair process. In the first stage, hMTH1 is responsible for oxidized purines hydrolyzation. It hydrolyzes 8-oxo-2’-deoxyguanosine-5’-triphosphate (8-oxo-dGTP) or 8-oxo-2’-deoxyguanosine-5’-diphosphate (8-oxo-dGDP) to 8-oxo-2’-deoxyguanosine-5’-monophosphate (8-oxo-dGMP), which are not substrates in DNA polymerization [65].

7. Concentration of 8-OHdG/8-oxodG and Risk of GDM Development

There is no clear evidence of a relationship between DNA damage and risk of glucose intolerance in pregnancy or GDM development. Nonetheless, has been observed is that among patients with GDM or mild gestational hyperglycemia (MGH), a higher level of oxidative DNA damage is presented (Table 1).
Table 1. DNA damage in hyperglycemic state during pregnancy.

| Type of Diabetes                          | Study Type   | Sample                | Result                                      | Reference |
|------------------------------------------|--------------|-----------------------|---------------------------------------------|-----------|
| MGH diabetic women with obesity and hypertension | Clinical     | Maternal lymphocytes  | ↑ Overall oxidative DNA damage              | [66]      |
| GDM                                      | Clinical     | Maternal lymphocytes  | ↑ 8-oxoG                                    |           |
| MGH                                      | Clinical     | Maternal urine        | ↑ 8-OHdG                                    |           |
| GDM                                      | Clinical     | Maternal urine        | ↑ 8-OHdG                                    | [20]      |
| GDM                                      | Clinical     | Maternal lymphocytes  | ↑ 8-oxoG                                    | [67]      |
| GDM                                      | Clinical     | Maternal urine        | ↑ 8-OHdG                                    |           |
| MGH, induced by streptozotocin            | Experimental | Maternal and fetal leukocytes | ↑ 8-OHdG, ↑ 8-oxo-dG | [68]      |
| Severe, induced by streptozotocin         | Experimental | Maternal leukocytes   | ↑ Overall oxidative DNA damage              | [69]      |
| Severe, induced by streptozotocin         | Experimental | Maternal leukocytes   | ↑ Overall oxidative DNA damage              |           |
| Severe, induced by streptozotocin         | Experimental | Fetal leukocytes      | ↑ Overall oxidative DNA damage              | [70]      |

GDM: Gestational Diabetes Mellitus; MGH: Mild Gestational Hyperglycemia.

There is growing evidence which indicates that a high level of oxidized purines reflects DNA damage triggered by hyperglycemia. Among patients with MGH (characterized by hyperglycemia, high insulin resistance indices (HOMA-IR), obesity and hypertension) elevated levels of oxidized pyrimidines are recorded [66]. According to Collins et al. [67], the results suggest that various enzymes react differently with oxidized nucleobases (purines or pyrimidines) in various conditions. Formamidopyrimidine DNA glycosylase (FPG) sensitive sites, are increased in women with higher glycemic levels, but not in women with MGH. Patients with MGH, obesity, hypertension, and hyperglycemia presented higher levels of endonuclease III sensitive sites. An increased level of endonuclease III sensitive (Endo III)-sites reflect an overall oxidative damage of DNA, probably induced by hypertension and other side effects of diabetes, whereas FPG-sensitive sites indicate specific damage—8-oxo-guanine triggered by hyperglycemia [66,67]. The results obtained by Gelaleti et al. [66] show that the oxidative DNA damage level (oxidized purines) in lymphocytes of women with GDM was about 70%, whereas the equivalent among the control group was about 65%, with statistical significance \( p < 0.05 \). Furthermore, the urine concentration of 8-OHdG in GDM women and MGH was marginally higher than in the control group, however, with no statistical significance \( p > 0.05 \).

In a pilot study Qiu et al. [20] revealed that an increased level of urine 8-OHdG concentrations correlated with a higher risk of GDM development. Moreover, the level of 8-OHdG in urine among patients with a GDM total of >8.01 ng/mg of creatinine and risk of developing GDM was 3.79-fold higher than for women whose concentration of 8-OHdG in urine was <4.23 ng/mg creatinine.

Apart from that, the intracellular oxidative status in women during pregnancy also correlated with the fate of a fetus. It seems that a fetal organism is more immune to DNA damage and cell apoptosis induced by oxidative stress than a mother. However, it is possible that there is a limit beyond which the fate of the fetus is precipitously changed [71].

Animal studies confirm that general DNA damage in leukocytes from diabetic female rats (mothers) and their fetuses was higher in comparison to control group [68–70]. Moreover, Lima et al. [68] reported that (i) among rats with severe diabetes during pregnancy hyperglycemia induced oxidative DNA damage which was identified by FPG or Endo III enzymes, (ii) offspring from rats with severe diabetes presented a higher level of 8OHdG and/or 8-oxo-dG, (iii) rats with mild diabetes and their newborns...
presented a higher level of 8OHdG and/or 8-oxodG, which can confirm the fact that FPG sensitive sites do not depend on oxidative stress but instead on induced hyperglycemia. These results may suggest that oxidative DNA damage can reflect concentration of blood glucose and intensity of diabetes mellitus. Therefore, previous observations allow us to conclude that GDM is a very early stage of diabetes which may evolve into T2DM in the future [4].

8. Maintenance of Genomic Integrity during GDM

Currently, it is known that maternal hyperglycemia during pregnancy can lead to the formation of oxidative DNA modification, mainly in maternal genetic material [71]. In order to protect and maintain DNA stability, organisms develop a cellular mechanism known as DNA damage response (DDR). Cells with incorrect DDR have a higher risk of mutagenesis, cell death, and diseases development, like cancer, neurodegenerative disorders, cardiovascular disease, and finally metabolic syndrome [72]. Studies confirm the importance of the DNA repair mechanism in pregnancy and fetus development. Among patients whose fetus had DNA repair diseases such as trichothiodystrophy (TTD) or xeroderma pigmentosum D (XPD), an increased risk to deliver preterm, or develop preeclampsia as well as HELLP (hemolysis, elevated liver enzymes, and low platelet count) syndrome was observed. Moreover, fetus movement was decreased [73]. Studies conducted on nonpregnant patients with T1DM and T2DM (both children and adults) showed that patients with T2DM have a higher level of oxidative DNA lesions and decreased efficiency of DDR mechanism compared with other diabetic subjects. Moreover, children with T1DM have a reduced level of DNA and increased efficiency of DDR mechanism [74]. These findings indicate differences in the efficiency of DNA repair mechanism between T1DM and T2DM subjects, which can be associated with long-term consequences. It suggests that the damaging effect of hyperglycemia can be reversed by using DNA repair mechanism. Nonetheless, disturbance in this mechanism can lead to the abnormal maternal-fetal interface [71].

The clinical intervention strategy used to maintain optimal glucose level is based on lifestyle changes including the diet therapy (eating regular meals with low glycemic index (LGI), avoid drinks and food with sugar) and increased physical activity [6,75]. The important thing in GDM treatment is also self-monitoring glucose (fasting glucose and after 1 and 2 h as well as after every meal) [75]. There are different recommendations for nonpregnant diabetics and patients with GDM. According to the American Diabetes Association (ADA), the maternal fasting glucose concentration for the latter should be maintained at the following thresholds values: <95 mg/dL, <140 mg/dL after 1-h, or <120 mg/dL after 2-h [76]. Apart from that, some research suggest that high-dose supplementation of antioxidants in pregnant diabetic rats can regulate the development of their newborns but this treatment is not recommended for nondiabetic pregnancy [77]. Collected evidence indicates that vitamin D can increase insulin secretion and reduce systemic inflammation [78]. Moreover, supplementation of vitamin C during pregnancy can induce preterm delivery but not stillbirth or perinatal death [79]. When lifestyle changes will not provide the expected results, then insulin administration is required. However, this option is only recommended for pregnant women who struggle to maintain the right glucose concentration level [80]. Results obtained by Brown et al. suggest that insulin administration can cause a higher risk of hypertensive disorders development and probably preterm delivery [81]. There is still no clear evidence for the effect of insulin and antioxidant treatment on plasma 8-OHG and tissue 8-OHdG [82]. Despite this fact, controlling of glucose concentration seems to be the most important factor in DNA protection not only because of diabetes and associated with its deleterious effects, but also because of the possibility of developing cancer [83].

9. 8-OHdG/8-oxodG as a Potential Biomarker for GDM—Summary

Gestational diabetes mellitus is associated with numerous health complications for a mother and her fetus. Although the mechanism of GDM development remains unclear, oxidative stress seems to be an important factor contributing to development of GDM. Current evidence suggests that even a mild form of hyperglycemia can induce oxidative damage of maternal DNA, which may be observed
as an elevated level of 8-OHdG/8-oxodG. Moreover, a higher level of 8-OHdG/8-oxodG alteration in early gestation can be an important factor stimulating gestational diabetes mellitus development, whereas a high level of 8-OHdG or 8-oxodG, which remains after pregnancy, can induce type 2 diabetes mellitus development later. Based on novel data, analysis of urine 8-OHdG concentration can be an important biomarker of GDM mainly due to utilizing statistically significant results, providing an easy way to obtain a test sample, and the fact that urinary excretion of 8-OHdG is not associated with human diet. According to a study performed by Qiu et al. [20], 8-OHdG concentrations of ≥8.01 ng/mg creatinine can be probably a significant indicator of oxidative stress and consequently, of higher risk of GDM development. However, this possible cut-off value should be more closely examined before later clinical application, and should be integrated with insulin sensitivity indices.

Therefore, future studies should focus on deepening knowledge on 8-hydroxy-2′-deoxyguanosine/8-oxo-7,8-dihydro-2′-deoxyguanosine as a potential biomarker for patients with mild gestational hyperglycemia, before GDM development. To achieve a better understanding of the etiology of this disease and its pathogenesis, it is necessary to select high-risk patients and examine the correlation between concentration of 8OHdG/8-oxodG alteration and progression from gestational diabetes mellitus to type 2 diabetes mellitus.

Author Contributions: Conceptualization, S.K.U.; Methodology, n/a.; Software, n/a.; Validation, n/a.; Formal Analysis, n/a.; Investigation, n/a.; Resources, n/a.; Data Curation, n/a.; Writing-Original Draft Preparation, n/a.; Writing-Review & Editing, S.K.U., K.B., M.S., J.K.-B. and B.T.K.; Visualization, S.K.U. and B.T.K.; Supervision, B.T.K.; Project Administration, n/a.; Funding Acquisition, B.T.K. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the Medical University of Lodz (503/3-045-02/503-31-002) and by the National Science Center, Poland (grant No. 2016/23/B/NZ7/03367).

Conflicts of Interest: The authors declare no conflict of interest.

References
1. Badakhsh, M.; Balouchi, A.; Amirshahi, M.; Hashemi, Z. Gestational diabetes and its maternal and neonatal complications: A review article. IJPT 2016, 8, 18868–18878.
2. Pikee, S.; Sakshi, M.; Aruna, N. Screening and Diagnosis of Gestational Diabetes Mellitus: From Controversy to Consensus. CRDOJ 2017, 2, 55600.
3. Roglic, G. WHO Global report on diabetes: A summary. Int. J. Non-Commun. Dis. 2016, 1, 3–8. [CrossRef]
4. Law, K.P.; Zhang, H. The pathogenesis and pathophysiology of gestational diabetes mellitus: Deductions from a three-part longitudinal metabolomics study in China. Clin. Chim. Acta 2017, 468, 60–70. [CrossRef]
5. Ben-Haroush, A.; Yoge, Y.; Hod, M. Epidemiology of gestational diabetes mellitus and its association with Type 2 diabetes. Diabet. Med. 2004, 21, 103–113. [CrossRef]
6. Kampmann, U.; Madsen, L.R.; Skajaa, G.O.; Iversen, D.S.; Moeller, N.; Ovesen, P. Gestational diabetes: A clinical update. World J. Diabetes 2015, 6, 1065–1072. [CrossRef]
7. HAPO Study Cooperative Research Group; Metzger, B.E.; Lowe, L.P.; Dyer, A.R.; Trimble, E.R.; Chaovarindr, U.; Coustan, D.R.; Hadden, D.R.; McCance, D.R.; Hod, M.; et al. Hyperglycemia and adverse pregnancy outcomes. N. Engl. J. Med. 2008, 358, 1991–2002.
8. Cypryk, K.; Szymczak, W.; Czupryniak, L.; Sobczak, M.; Lewiński, A. Gestational diabetes mellitus—An analysis of risk factors. Endokrynol. Pol. 2008, 59, 393–397.
9. Kaaja, R.; Rönnemaa, T. Gestational diabetes: Pathogenesis and consequences to mother and offspring. Rev. Diabet. Stud. 2008, 5, 194–202. [CrossRef]
10. Shah, B.R.; Retnakaran, R.; Booth, G.L. Increased risk of cardiovascular disease in young women following gestational diabetes mellitus. Diabetes Care 2008, 31, 1668–1669. [CrossRef]
11. Sathiamma, P.K.; Karunakaran, L. A prospective study on maternal and perinatal outcome of gestational diabetes mellitus. Int. J. Reprod. Contracept. Obstet. Gynecol. 2017, 6, 2933–2938.
12. Saiho, Y.; Miyakoshi, K.; Tanaka, M.; Shimada, A.; Ikenoue, S.; Kadohira, I.; Yoshimura, Y.; Itoh, H. Beta cell dysfunction and its clinical significance in gestational diabetes. Endocr. J. 2010, 57, 973–980. [CrossRef] [PubMed]
13. Kuzmicki, M.; Telejko, B.; Zonenberg, A.; Szamatowicz, J.; Kretowski, A.; Nikolajuk, A.; Laudanski, P.; Gorska, M. Circulating pro- and anti-inflammatory cytokines in Polish women with gestational diabetes. *Horm. Metab. Res.* 2008, 40, 556–560. [CrossRef] [PubMed]

14. Hotamisligil, G.S. Endoplasmic reticulum stress and the inflammatory basis of metabolic disease. *Cell* 2010, 140, 900–917. [CrossRef] [PubMed]

15. Barbour, L.A.; McCurdy, C.E.; Hernandez, T.L.; Kirwan, J.P.; Catalano, P.M.; Friedman, J.E. Cellular mechanisms for insulin resistance in normal pregnancy and gestational diabetes. *Diabetes Care* 2007, 30 (Suppl. 2), S112–S119. [CrossRef] [PubMed]

16. Wójcik, M.; Chmielewska-Kassassir, M.; Grzywnowicz, K.; Wozniak, L.; Cypryk, K. The relationship between adipose tissue-derived hormones and gestational diabetes mellitus (GDM). *Endokrynol. Pol.* 2014, 65, 134–142. [CrossRef]

17. Lappas, M.; Hiden, U.; Desoye, G.; Froehlich, J.; Hauguel-de Mouzon, S.; Jawerbaum, A. The role of oxidative stress in the pathophysiology of gestational diabetes mellitus. *Antioxid. Redox Signal.* 2011, 15, 3061–3100. [CrossRef]

18. Cadet, J.; Wagner, J.R.; Shafirovich, V.; Geacintov, N.E. One-electron oxidation reactions of purine and pyrimidine bases in cellular DNA. *Int. J. Radiat. Biol.* 2014, 90, 423–432. [CrossRef]

19. Valavanidis, A.; Vlachogianni, T.; Fiotakis, C. 8-hydroxy-2′-deoxyguanosine (8-OHdG): A critical biomarker of oxidative stress and carcinogenesis. *J. Environ. Sci. Health C Environ. Carcinog. Ecotoxicol. Rev.* 2009, 27, 120–139. [CrossRef]

20. Qiu, C.; Hevner, K.; Abetew, D.; Enquobahrie, D.A.; Williams, M.A. Oxidative DNA damage in early pregnancy and risk of gestational diabetes mellitus: A pilot study. *Clin. Biochem.* 2011, 44, 804–808. [CrossRef]

21. Qiu, C.; Hevner, K.; Abetew, D.; Sedensky, M.; Morgan, P.; Enquobahrie, D.A.; Williams, M.A. Mitochondrial DNA copy number and oxidative DNA damage in placental tissues from gestational diabetes and control pregnancies: A pilot study. *Clin. Lab.* 2013, 59, 655–660. [CrossRef] [PubMed]

22. Nita, M.; Grzybowski, A. The Role of the Reactive Oxygen Species and Oxidative Stress in the Pathomechanism of the Age-Related Ocular Diseases and Other Pathologies of the Anterior and Posterior Eye Segments in Adults. *Oxid. Med. Cell. Longev.* 2016, 2016, 3164734. [CrossRef] [PubMed]

23. Lindahl, T. Instability and decay of the primary structure of DNA. *Nature* 1993, 362, 709–715. [CrossRef] [PubMed]

24. Kalisz, O.; Wolski, T.; Gerkowicz, M.; Smorawski, M. Reaktywne formy tlenu (RFT) oraz ich rola w patogenezie niektórych chorób. *Ann. Univ. Mariae Curie-Skłodowska Sect. DD Med. Vet.* 2007, 62, 87–99.

25. Sies, H. Oxidative stress: Oxidants and antioxidants. *Exp. Physiol.* 1997, 82, 291–295. [CrossRef]

26. Turek, I.A.; Wozniak, L.A.; Cypryk, K.; Wójcik, M. Hyperglycemia-induced oxidative stress in gestational diabetes mellitus (GDM). *Diabetol. Prakt.* 2015, 4, 189–198.

27. Nowotny, K.; Jung, T.; Höhn, A.; Weber, D.; Grune, T. Advanced glycation end products and oxidative stress in type 2 diabetes mellitus. *Biomolecules* 2015, 5, 194–222. [CrossRef]

28. Unoki, H.; Yamamoto, H.; Kugiyama, K.; Yasue, H.; Miyamoto, E. Involvement of protein kinase C in superoxide anion-induced activation of nuclear factor-kappa B in human endothelial cells. *Cardiovasc. Res.* 2000, 5, 513–521. [CrossRef]
34. Eitel, K.; Staiger, H.; Rieger, J.; Mischak, H.; Brandhorst, H.; Brendel, M.D.; Bretzel, R.G.; Häring, H.U.; Kellerer, M. Protein kinase C delta activation and translocation to the nucleus are required for fatty acid-induced apoptosis of insulin-secreting cells. *Diabetes* 2003, 52, 991–997. [CrossRef]

35. Safi, S.Z.; Qvist, R.; Kumar, S.; Batumalai, K.; Ismail, I.S. Molecular mechanisms of diabetic retinopathy, general preventive strategies, and novel therapeutic targets. *BioMed Res. Int.* 2014, 2014, 801269. [CrossRef]

36. Mathebula, S.D. Polyol pathway: A possible mechanism of diabetes complications in the eye. *Afr. Vis. Eye Health* 2015, 74, 5. [CrossRef]

37. Schleicher, E.D.; Weigert, C. Role of the hexosamine biosynthetic pathway in diabetic nephropathy. *Kidney Int. Suppl.* 2000, 77, S13–S18. [CrossRef]

38. Redza-Dutordoir, M.; Averill-Bates, D.A. Activation of apoptosis signalling pathways by reactive oxygen species. *Biochim. Biophys. Acta* 2016, 1863, 2977–2992. [CrossRef]

39. Birben, E.; Sahiner, U.M.; Sackesen, C.; Erzurum, S.; Kalayci, O. Oxidative stress and antioxidant defense. *World Allergy Organ. J.* 2012, 5, 9–19. [CrossRef]

40. Bernea, E.G.; Antohe, F.; Mihai, A.; Ionecsu-Tirgoviste, C. Oxidative stress and gestational diabetes mellitus. The effects of supplements on oxidative stress. *Proc. Rom. Acad. Ser. B* 2018, 20, 121.

41. Ho, E.; Galougahi, K.K.; Liu, C.C.; Bhindi, R.; Figtree, G.A. Biological markers of oxidative stress: Applications to cardiovascular research and practice. *Redox Biol.* 2013, 1, 483–491. [CrossRef] [PubMed]

42. Girotti, A.W. Mechanisms of lipid peroxidation. *J. Free Radic. Biol. Med.* 1985, 1, 87–95. [CrossRef]

43. Gutteridge, J.M. Lipid peroxidation and antioxidants as biomarkers of tissue damage. *Clin. Chem.* 1995, 41, 1819–1828. [PubMed]

44. Biri, A.; Onana, A.; Devrimb, E.; Babacanc, F.; Kavutcua, M.; Durakb, I. Oxidant Status in Maternal and Cord Plasma and Placental Tissue in Gestational Diabetes. *Placenta* 2006, 27, 327–332. [CrossRef] [PubMed]

45. Grissa, O.; Ategbo, J.M.; Yessoufou, A.; Tabka, Z.; Miled, A.; Djennabi, M.; Moutairou, K.; Prost, J.; Hichami, A.; et al. Antioxidant status and circulating lipids are altered in human gestational diabetes and macrosomia. *Transl. Res.* 2007, 150, 164–171. [CrossRef] [PubMed]

46. Bis-Ghuchowska, M.; Marciniak, B.; Szpringer-Bogu, E.; Rola, R.; Leszczyńska-Gorzela, B.; Oleszczuk, J. Determination of antioxidative-oxidative balance in the cord blood of newborns delivered to mothers with diabetes type G1. *Ginekol. Pol.* 2001, 72, 1255–1258.

47. Vural, M.; Camuzcuoglu, H.; Toy, H.; Cece, H.; Aydin, H.; Eren, M.A.; Kocyigit, A.; Aksoy, N. Evaluation of the future atherosclerotic heart disease with oxidative stress and carotid intima media thickness in gestational diabetes mellitus. *Endocr. Res.* 2012, 37, 145–153. [CrossRef]

48. Coughlan, M.T.; Vervaart, P.P.; Permezel, M.; Georgiou, H.M.; Rice, G.E. Altered placental oxidative stress status in gestational diabetes mellitus. *Placenta* 2004, 25, 78–84. [CrossRef]

49. Singh, V.P.; Bali, A.; Singh, N.; Jaggi, A.S. Advanced glycation end products and diabetic complications. *Korean J. Physiol. Pharmacol.* 2014, 18, 1–14. [CrossRef]

50. Karacay, O.; Sepici-Dincel, A.; Karacaaltincaba, D.; Sahin, D.; Yalvaç, S.; Akyol, M.; Kandemir, O.; Altan, N. A quantitative evaluation of total antioxidant status and oxidative stress markers in preeclampsia and gestational diabetic patients in 24-36 weeks of gestation. *Diabetes Res. Clin. Pract.* 2010, 89, 231–238. [CrossRef]

51. Gelisgen, R.; Genc, H.; Kayali, R.; Oncul, M.; Benian, A.; Guralp, O.; Uludag, S.; Cakatay, U.; Albayrak, M.; Uzun, H. Protein oxidation markers in women with and without gestational diabetes mellitus: A possible relation with paraoxonase activity. *Diabetes Res. Clin. Pract.* 2011, 94, 404–409. [CrossRef] [PubMed]

52. Georgiou, H.M.; Lappas, M.; Georgiou, G.M.; Marita, A.; Bryant, V.J.; Hiscock, R.; Permezel, M.; Khalil, Z.; Rice, G.E. Screening for biomarkers predictive of gestational diabetes mellitus. *Acta Diabetol.* 2008, 45, 157–165. [CrossRef] [PubMed]

53. López-Tinoco, C.; Roca, M.; García-Valero, A.; Murri, M.; Tinahones, F.J.; Segundo, C.; Bartha, J.L.; Aguilar-Diosdado, M. Oxidative stress and antioxidant status in patients with late-onset gestational diabetes mellitus. *Acta Diabetol.* 2013, 50, 201–208. [CrossRef] [PubMed]

54. Suhail, M.; Patil, S.; Khan, S.; Siddiqui, S. Antioxidant Vitamins and Lipoperoxidation in Non-pregnant, Pregnant, and Gestational Diabetic Women: Erythrocytes Osmotic Fragility Profiles. *J. Clin. Med. Res.* 2010, 2, 266–273. [CrossRef] [PubMed]

55. Santra, D.; Sawhney, H.; Aggarwal, N.; Majumdar, S.; Vasishtha, K. Lipid peroxidation and vitamin E status in gestational diabetes mellitus. *J. Obstet. Gynaecol. Res.* 2003, 29, 300–304. [CrossRef]
56. Ba, X.; Boldogh, I. 8-Oxoguanine DNA glycosylase 1: Beyond repair of the oxidatively modified base lesions. *Redox Biol.* 2018, 14, 669–678. [CrossRef]

57. Cadet, J.; Douki, T.; Ravanan, J.L. Oxidatively generated base damage to cellular DNA. *Free Radic. Biol. Med.* 2010, 49, 9–21. [CrossRef]

58. Ghosh, R.; Mitchell, D.L. Effect of oxidative DNA damage in promoter elements on transcription factor binding. *Nucleic Acids Res.* 1999, 27, 3213–3218. [CrossRef]

59. Song, J.; Pfeifer, G.P. Are there specific readers of oxidized 5-methylcytosine bases? *BioEssays* 2016, 38, 1038–1047. [CrossRef]

60. Marietta, C.; Gulam, H.; Brooks, P.J. A single 8,5′-cyclo-2′-deoxyadenosine lesion in a TATA box prevents binding of the TATA binding protein and strongly reduces transcription in vivo. *DNA Repair* 2002, 1, 967–975. [CrossRef]

61. Cadet, J.; Douki, T.; Ravanan, J.L. The human genome as a target of oxidative modification: Damage to nucleic acids. In *Redox-Genome Interactions in Health and Disease*; Fuchs, J., Podda, M., Packer Marcel Dekker, L., Eds.; CRC Press: New York, NY, USA, 2003; pp. 145–192.

62. Simone, S.; Gorin, Y.; Velagapudi, C.; Abboud, H.E.; Habib, S.L. Mechanism of oxidative DNA damage in diabetes: Tuberin inactivation and downregulation of DNA repair enzyme 8-oxo-7,8-dihydro-2′-deoxyguanosine-DNA glycosylase. *Diabetes 2008*, 57, 2626–2636. [CrossRef] [PubMed]

63. Lindahl, T. An N-glycosidase from Escherichia coli that releases free uracil from DNA containing deaminated cytosine residues. *Proc. Natl. Acad. Sci. USA* 1974, 71, 3649–3653. [CrossRef] [PubMed]

64. Akiyama, M.; Maki, H.; Sekiguchi, M.; Horiuchi, T. A specific role of MutT protein: To prevent dG.dA mismatching in DNA replication. *Proc. Natl. Acad. Sci. USA* 1989, 86, 3949–3952. [CrossRef] [PubMed]

65. Nakabeppu, Y.; Ohta, E.; Abolhassani, N. MTH1 as a nucleotide pool sanitizing enzyme: Friend or foe? *Free Radic. Biol. Med.* 2017, 107, 151–158. [CrossRef] [PubMed]

66. Gelaleti, R.B.; Damasceno, D.C.; Lima, P.H.; Salvadori, D.M.; Calderon, I.M.; Peraçoli, J.C.; Rudge, M.V. Oxidative DNA damage in diabetic and mild gestational hyperglycemic pregnant women. *Diabetol. Metab. Syndr.* 2015, 7, 1. [CrossRef]

67. Collins, A.R.; Raslová, K.; Smorovská, M.P.; Petrovská, H.; Ondrusová, A.; Vohnout, B.; Fáby, R.; Dusinská, M. DNA damage in diabetes: Correlation with a clinical marker. *Free Radic. Biol. Med.* 1998, 25, 373–377. [CrossRef]

68. Lima, P.H.; Sinzato, Y.K.; Gelaleti, R.B.; Calderon, I.M.; Rudge, M.V.; Damasceno, D.C. Genotoxicity evaluation in severe or mild diabetic pregnancy in laboratory animals. *Exp. Clin. Endocrinol. Diabetes 2012*, 120, 303–307. [CrossRef]

69. Lim, P.H.; Sinzato, Y.K.; de Souza Mda, S.; Braz, M.G.; Rudge, M.V.; Damasceno, D.C. Evaluation of level of DNA damage in blood leukocytes of non-diabetic and diabetic rat exposed to cigarette smoke. *Mutat. Res.* 2007, 628, 117–122. [CrossRef]

70. Liao, P.; Damasceno, D.C.; Sinzato, Y.K.; de Souza Mda, S.; Salvadori, D.M.; Calderon, I.M.; Rudge, M.V. Levels of DNA damage in blood leukocyte samples from non-diabetic and diabetic female rats and their fetuses exposed to air or cigarette smoke. *Mutat. Res.* 2008, 653, 44–49. [CrossRef]

71. Morelli, J.B.; Santos, J.H.; Lorenzon-Ojea, A.R.; Corrêa-Silva, S.; Fortunato, R.S.; Rocha, C.R.; Rudge, M.V.; Damasceno, D.C.; Bevilacqua, E.; Calderon, I.M. Hyperglycemia Differentially Affects Maternal and Fetal DNA Integrity and DNA Damage Response. *Int. J. Biol. Sci.* 2016, 12, 466–477. [CrossRef]

72. Giglia-Mari, G.; Zotter, A.; Vermeulen, W. DNA damage response. *Cold Spring Harb. Perspect. Biol.* 2011, 3, a000745. [CrossRef] [PubMed]

73. Mosleh, R.; Signore, C.; Tamura, D.; Mills, J.L.; Digiovanna, J.J.; Tucker, M.A.; Troedle, J.; Ueda, T.; Boyle, J.; Khan, S.G.; et al. Adverse effects of trichothiodystrophy DNA repair and transcription gene disorder on human fetal development. *Clin. Genet.* 2010, 77, 365–373. [CrossRef] [PubMed]

74. Pácal, L.; Varvařovská, J.; Rušavý, Z.; Lacigová, S.; Stětina, R.; Rasek, J.; Pomahačová, R.; Tanháuserová, V.; Kaňková, K. Parameters of oxidative stress, DNA damage and DNA repair in type 1 and type 2 diabetes mellitus. *Arch Physiol. Biochem.* 2011, 117, 222–230. [CrossRef] [PubMed]

75. Metzger, B.E.; Buchanan, T.A.; Coustan, D.R.; de Leiva, A.; Dunger, D.B.; Hadden, D.R.; Hod, M.; Kitzmiller, J.L.; Kjos, S.L.; Oats, J.N.; et al. Summary and recommendations of the Fifth International Workshop-Conference on Gestational Diabetes Mellitus. *Diabetes Care* 2007, 30 (Suppl. 2), S251–S260. [CrossRef]
76. American Diabetes Association (ADA). Standards of medical care in Diabetes. *Diabetes Care* 2014, 37, S14–S80. [CrossRef]
77. Cederberg, J.; Eriksson, U.J. Antioxidative treatment of pregnant diabetic rats diminishes embryonic dysmorphogenesis. *Birth Defects Res. A Clin. Mol. Teratol.* 2005, 73, 498–505. [CrossRef]
78. Poel, Y.H.; Hummel, P.; Lips, P.; Stam, F.; van der Ploeg, T.; Simsek, S. Vitamin D and gestational diabetes: A systematic review and meta-analysis. *Eur. J. Intern. Med.* 2012, 23, 465–469. [CrossRef]
79. Rumbold, A.; Ota, E.; Nagata, C.; Shahrook, S.; Crowther, C.A. Vitamin C supplementation in pregnancy. *Cochrane Database Syst. Rev.* 2015, 9. [CrossRef]
80. Rowan, J.A.; Hague, W.M.; Gao, W.; Batin, M.R.; Moore, M.P. Metformin versus insulin for the treatment of gestational diabetes. *N. Engl. J. Med.* 2008, 358, 2003–2015. [CrossRef]
81. Brown, J.; Grzeskowiak, L.; Williamson, K.; Downie, M.R.; Crowther, C.A. Insulin for the treatment of women with gestational diabetes. *Cochrane Database Syst. Rev.* 2017, 11. [CrossRef]
82. Park, K.S.; Kim, J.H.; Kim, M.S.; Kim, J.M.; Kim, S.K.; Choi, J.Y.; Chung, M.H.; Han, B.; Kim, S.Y.; Lee, H.K. Effects of insulin and antioxidant on plasma 8-hydroxyguanine and tissue 8-hydroxy-2’-deoxyguanosine in streptozotocin-induced diabetic rats. *Diabetes* 2001, 50, 2837–2841. [CrossRef] [PubMed]
83. Hou, Y.; Zhou, M.; Xie, J.; Chao, P.; Feng, Q.; Wu, J. High glucose levels promote the proliferation of breast cancer cells through GTPases. *Breast Cancer* 2017, 9, 429–436. [CrossRef] [PubMed]

© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).