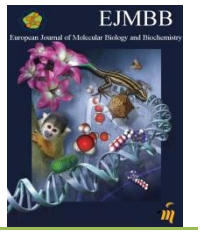
e - ISSN - 2348-2206
Print ISSN - 2348-2192

European Journal of Molecular Biology and Biochemistry

Journal homepage: www.mcmed.us/journal/ejmbb



EVALUATION OF ENDOGENOUS ANTIOXIDANT STATUS OF PREGNANT DOG

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Article Info

Received 28/03/2016
Revised 10/04/2016
Accepted 15/04/2016

Key words:-
Antioxidants, German Shepherd, oxidative stress, pregnant.

ABSTRACT

The present investigation was carried out to evaluate endogenous antioxidant status of pregnant dogs. Sera were obtained to determine endogenous antioxidants like vitamin A, C, E and glutathione in healthy pregnant and non pregnant adult female German Shepherd dogs. Results exhibited significant ($p \leq 0.05$) decrease in the values of serum vitamin A, C, E and glutathione during pregnancy as compared to non pregnant state. It indicated depletion of antioxidant status of pregnant dogs. It signified the development of oxidative stress in pregnant dogs. Greatest percent change was observed in the mean value of serum glutathione. The present investigation has tried to evaluate the relationship of antioxidants with the oxidative stress. It can be concluded that pregnancy produced the oxidative stress in the dogs. Interpretation of these variations will help to provide perceptive approach of various hidden mechanisms in the development of oxidative stress. Results undoubtedly demonstrated that apart from diseases, other stressors can also be a factor in the development of oxidative stress and pregnancy is one of them. Canines must be supplemented with antioxidants to enhance endogenous antioxidant status to safeguard them from the danger of oxidative stress throughout pregnancy.

INTRODUCTION

Pregnancy is a physiological period during which different metabolic pathways are altered, resulting in greater oxygen consumption and modifications of the consumption of energy substrates, with a consequent greater exposure to oxidative stress [1]. Oxidative stress is generally described as an imbalance between reactive oxygen species within cells. The most important antioxidants include vitamins A, C and E, which work by slowing or stopping the development of reactive oxygen in cells. Recent studies have focused on the role of oxidative stress and pregnancy complications. Oxidative stress can cause spontaneous abortions, as well as a host of female reproductive disorders. It is also associated with the development of premature rupture of foetal membranes, causing stillborn and those born with severe birth defects and developmental disabilities.Disturbances in normal cellular growth and development can cause toxic effects throughout the body. The body has a natural detoxification system, mainly composed of glutathione and essential amino acids. Glutathione, as the body's natural detoxifier, helps remove harmful chemicals and also prevents certain diseases from infiltrating healthy cells. Free radicals are described as a group of atoms with an unpaired number of electrons. They are developed within cells when oxygen interacts with certain molecules. As they develop, free radicals form a chain reaction, causing cellular damage. Cells eventually die from an influx of free radicals, often leading to serious infections and diseases. Free radicals can cause cellular damage, as well as irreparable damage to

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RNA and DNA synthesis, both of which can cause fertility disorders and death to a developing foetus. The antioxidant effects of glutathione stop the cascading effects of free radicals, helping maintain cellular health. An important aspect in fertility, antioxidants help neutralize free radicals, preventing permanent cellular damage, especially in the female reproductive system. Glutathione works as a natural defense system, eliminating free radicals before they can cause cellular damage. High levels of glutathione have been shown to help the female reproductive system operate properly, producing eggs and increasing the chance of conception and maintaining a healthy pregnancy. Although many prenatal vitamins are usually prescribed in human medicine in the early stages of pregnancy, many clinicians are not aware of glutathione and its positive effects in promoting fertility. Interferences in the maternal environment are known to reprogram the offspring metabolism response, impacting in the risk of chronic diseases development in adulthood. Intrauterine food restriction may disrupt oxidative status, impairing the antioxidant network. Studies carried out in dams and pups showed oxidative modulation caused by food restriction in cerebellum and cerebral cortex. Brain structures are affected concerning to catalase, glutathione peroxidase and glutaredoxin activities [2].

Good nutrition and a balanced diet are essential elements for good health in a dog. This is especially true in the pregnant female dog. Overfeeding or underfeeding at certain times can be detrimental to her health and the health of the developing puppies. Vitamin E promotes fertility, prevents cataracts, delays the aging process, regulates digestion, and supports cellular and respiratory health in dogs. In human medicine, preeclampsia (PE) is a pregnancy-specific disorder defined as new onset of maternal hypertension and proteinuria. Researchers have shown increased maternal oxidative stress at delivery to be associated with poor birth outcome in PE. To understand the role of antioxidant defense mechanisms in PE right from early pregnancy, measurement of superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione (GSH) concentrations in maternal blood becomes important. Research demonstrated that women who develop PE exhibit increased oxidative stress right from 16 to 20 weeks of gestation. This may alter placental development and lead to fetal programming of adult non-communicable disease in the offspring [3].

All through the last decade acceptance has become far and wide that pet ownership and animal assistance in therapy and education may have a throng of optimistic consequences on humans [4]. Dogs of German shepherd breed are dependable and good bodyguards. Consequently they are kept as companions by large number of families. Hence it becomes crucial to keep the pets stress free and well-timed uncovering of stress can strengthen the measures which help in preventing the incidence of diseases. Rareness of literature on this viewpoint in the dogs was adequate to embark on the initiation of a project with comprehensible directions to assess oxidative stress in pregnant dogs.

MATERIALS AND METHODS

The present endeavor was undertaken in twenty healthy adult (between 3 and 5 years) German Shepherd female pregnant (10) and non pregnant (10) dogs kept by the private owners with almost similar type of management conditions. These animals were free from endo- and ecto-parasites as assessed by routine faecal and skin examination, respectively. Blood sample were collected in sterile tubes to harvest sera during morning hours. Pregnant animals were in mid gestation phase. Serum vitamin A, vitamin E, vitamin C and glutathione were determined.

Serum vitamin A was determined by the methods as described by Varley [5] with little modification [6]. Proteins are precipitated with alcohol and the retinol and carotenes extracted into light petroleum. After reading the intensity of yellow colour due to carotenes, the light petroleum is evaporated and the residue is dissolved in chloroform before carrying out colour reaction.

Serum vitamin E was determined by the spectrophotometric method of Nair and Magar [7] with little modification. The method is highly sensitive and can detect the very small concentration of vitamin E in the blood. It is based upon the colour reaction between phosphomolybdic acid and vitamin E. Serum vitamin C was determined by the method as described by Varley [5]. This method is based upon the titration of serum ascorbate by 2,6-dichlorophenolindophenol dye. Serum glutathione was determined by the rapid colorimetric micro method of Owens and Belcher [8] with modifications for serum samples. Mean value of healthy non pregnant dogs for each parameter was considered as control.

RESULTS

The mean±SEM values of serum vitamin A, vitamin E, vitamin C and glutathione along with per cent variations in the values in pregnant and non pregnant dogs are presented in table 1. All the comparisons for each parameter during pregnancy have been made from respective non pregnant value, keeping it as control. Results indicated significant ($p \leq 0.05$) decrease in the levels of serum vitamin A, vitamin E, vitamin C and glutathione during pregnancy as compared to non pregnant state. Greatest percent change was observed in the mean value of serum glutathione.

Superscript ‘b’ indicates that a given parameter differs significantly ($p \leq 0.05$) from respective non pregnant mean value.

Table 1. Serum levels of antioxidants in non pregnant and pregnant dogs

Serum antioxidants	Female dogs		% Change
	Non pregnant	Pregnant	
Vitamin A, µmol L^{-1}	1.89 ± 0.01	1.21 ^{b} ± 0.01	35.97
Vitamin C µmol L^{-1}	26.00 ± 0.91	18.31 ^{b} ± 0.76	29.57
Vitamin E µmol L^{-1}	6.71 ± 0.13	5.09 ^{b} ± 0.11	24.14
Glutathione µmol L^{-1}	4.81 ± 0.05	3.03 ^{b} ± 0.04	37.00

(n=10, mean ± SEM values)

DISCUSSION

There is paucity of literature regarding information on serum antioxidant level in the dogs of German Shepherd breed during pregnancy. Highest percent change was observed in the mean value of serum glutathione indicating the development of oxidative stress.

Vitamin A

The low vitamin A level in the present study in pregnant dogs could be due to its higher mobilization for various metabolic purposes[6]. It is well known that vitamin A is an essential nutrient for normal cellular function, including reproduction and development. Vitamin A deficiency is a worldwide problem of great magnitude. Vitamin A functions in the embryo begin soon after conception and continue throughout the life of all vertebrates [9]. Vitamin A is important in maintaining normal growth, regulating proliferation and differentiation of epithelial tissues and maintaining visual and reproductive functions [10]. Antioxidant role of vitamin A is gaining importance in scientific community to protect the body from the danger of oxidative stress. Many a times it has been observed that animals supplemented with vitamin A also show lower plasma level of vitamin A. Such cases clearly indicate towards the oxidative stress in which vitamin A is used to neutralize the free radicals [6]. It is believed that requirements for immunity are higher than for growth or reproduction. In humans, vitamin A stores are known to be positively associated with several measures of innate immune activity across a broad range of stress, suggesting that vitamin A enhances protection against diverse pathogens even at concentrations above those needed to maintain normal vision [11]. Low vitamin A in pregnant dogs denoted the development of oxidative stress.

Vitamin E

Pregnancy associated oxidative stress in reference to vitamin E has been discussed in animals [12, 13]. Vitamin E is also important in the management of stressed animals [14], therefore its use is suggested to combat oxidative stress [15]. Vitamin E helps to protect cell membranes. It is one of the important immunonutrients to include in pregnant animals' diet. Although vitamin E deficiency is rarely seen in healthy adults, however, for pregnant, insufficient dietary vitamin E may lead to complications such as pre-eclampsia and the foetus being born small. During the last month of pregnancy the puppies grow fast. They will need a lot of nutrients. At this time supplementation with immunonutrients is required. Vitamin E is also known to boost the immune system. The vitamin E deficient puppies start to develop muscle paralysis and muscle weakness in their limbs. A decrease muscle mass and increased sensitivity to pain is observed. Free radicals are unstable, damaged molecules that threaten a dog's immune system by attacking healthy cells and increasing the risk of various disorders. A powerful antioxidant like vitamin E is crucial in improving and supporting immune system health because it reduces the number of free radicals formed and prevents much of the damage that could lead to serious health problems. Vitamin E supplements included in dog's diet can fortify his immune system to help keep him healthy and strong for longer. Vitamin E is a neuroprotectant and a powerful antioxidant. It suppresses the effects of oxidative stress on foetal brain tissue damage [16]. Decreased serum vitamin E levels in pregnant dogs revealed its depletion to combat the free radicals and marked the development of oxidative stress in pregnant dogs.

Vitamin C

Although vitamin C is synthesized by the dog in its liver from glucose with the help of the enzyme L-gluconolactone oxidase, however, during stress periods its supplementation becomes mandatory. The role of vitamin C is well documents as an anti-oxidant protecting the body against oxidative stress [17]. Mechanism involves the conversion of L-ascorbate to its oxidised form L-dehydroascorbate, which can then be reduced back to the active L-ascorbate form in the body by enzymes and glutathione. Vitamin C is important in the synthesis of collagen which is important for growth and development. Vitamin C is also important for the proper function of immune system. Commercial dog foods contain vitamin C because of its antioxidant property. Reversible oxidation-reduction of ascorbic acid with dehydroascorbic acid is the most important chemical property of vitamin C and the basis for its known physiological activities and stabilities [18]. Ascorbic acid is also stabilized by the antioxidant enzymes superoxide dismutase and catalase [19], which require copper, zinc, manganese and iron. Major role of

vitamin C is that of water-soluble antioxidant, where its function is linked to that of the antioxidant enzymes, such as glutathione peroxidase and to vitamin E. The antioxidant role of vitamin C appears to be a common link in its role in the function and integrity of various cell types in the body, in detoxification functions and in the normal functioning of the adrenal glands, lungs, brain, eye and immune system. In keratinocytes, vitamin C contributes to counteract oxidative stress via transcriptional and post-translational mechanisms. Vitamin C can act directly by scavenging reactive oxygen species generated by stressors, prevent reactive oxygen species mediated cell damage by modulating gene expression, regulate keratinocyte differentiation maintaining a balanced redox state, promote cell cycle arrest and apoptosis in response to DNA damage. Decreased levels of vitamin C confirm the presence of oxidative stress because repletion is reported after supplementation of vitamin C [20]. Scientists [21] have recommended the use of antioxidants in the disease conditions causing oxidative stress.

Glutathione

Glutathione is known to be body's master antioxidant. Many pregnancy complications and birth defects have been linked to oxidative stress. During pregnancy it protects both mother and foetus from damaging effects of free radicals and oxidative stress. Pregnancy in itself creates a lot of free radicals. Antioxidants counteract them. Glutathione works in preventing birth defects. Glutathione is an antioxidant which protects cells from reactive oxygen species [22] and improves antioxidant capacity of blood [23]. In the placenta, GSH detoxifies pollutants before it reaches the developing foetus. Effect of physiological states on blood glutathione activity was discussed by earlier workers in animals [13]. Lower serum levels of glutathione in pregnant dogs suggested the presence of oxidative stress.

CONCLUSION

Lower serum levels of endogenous antioxidants confirmed the oxidative stress in the pregnant dogs. Pattern of changes in serum antioxidants supported the possible physiological modulations under the influence of probable increase in reactive oxygen species during pregnancy. It can be concluded that pregnancy produced the oxidative stress in the dogs. It can be avowed that apart from diseases, other stressors can also contribute in the development in oxidative stress and pregnancy is one of them. Canines must be supplemented with antioxidants regularly to protect them from the threats of oxidative stress particularly during pregnancy.

ACKNOWLEDGEMENTS

The authors are thankful to the dog owners for allowing collecting blood to be used in this study.

CONFLICT OF INTEREST

No interest

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