IJBPAS, May, 2018, 7(5): 831-840

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

ASSESSMENT OF UPSTREAM LEVELS OF LIPID PEROXIDATION AND INFLAMMATORY CYTOKINES IN PRETERM INFANTS SUFFERING FROM ANEMIA

MALIK A*, SULTANA M, WAQUAR S, QURA-TUL-AIN, ASHRAF MAB AND ALI Q

Institute of Molecular Biology and Biotechnology, University of Lahore, Lahore, Pakistan

***Corresponding Author Email: arifuaf@yahoo.com; Contact No: +92(0)321-8448196**

Received 25th Jan. 2018; Revised 26th Feb. 2018; Accepted 22nd March 2018; Available online 1st May 2018

DOI: https://doi.org/10.31032/IJBPAS/2018/7.5.4441

ABSTRACT

Blood samples of 100 preterm infants suffering from anemia were collected from Shaheed Benazir hospital Rawalpindi and samples of 100 normal infants were served as a control. MDA, GSH, CAT, GPx and NO were estimated spectrophotometrically and IL-6 and TNF-α was measured by using commercially available Elisa Kit. Significantly elevated level of oxidative stress biomarkers were measured in serum sample of preterm anemic infants as compared to full-term infants. The level of MDA and NO were significantly increased in preterm infants (2.29±0.95 nmol/ml and 42.26±3.88 µmol/L respectively) as compared to full-term infants (0.95±0.019 nmol/ml and 21.05±3.99 µmol/L respectively). The serum antioxidants CAT, SOD, GSH and GPx were recorded with the significant decline values 3.09±1.09 IU/L, 0.069±0.005 IU/ml, 5.26±1.55 µmol/L and 6.25±1.58 µmol/L respectively. However, the serum levels of cytokines IL-6 (7.16±2.16 pg/ml) and TNF-α (43.26±4.26 pg/ml) were significantly elevated in preterm infants suffering from anemia. Preterm infants suffer from anemia because of low iron intake and loss of blood due to physiological decline of hemoglobin levels and phlebotomy. During this condition, the lifespan of RBCs decline. The decrease level of iron is responsible to enhance the potential of oxidative stress that cause lipid peroxidation, diminished the levels of anti-oxidants and secrete inflammatory cytokines.

Keyword: Oxidative Stress, lipid peroxidation, MDA, IL-6, TNF-α, GSH, SOD, CAT

INTRODUCTION

The term and preterm labor are believed to be a primarily comparable process in which they both have same characteristics such as cervical dilation, uterine contraction and stimulation of amniotic membranes. This phenomenon includes biochemical, anatomical, clinical and endocrinological events which happen between the mother and fetus life. In worldwide, it is one of the most common causes of death in babies. Approximately, 15 million infants are preterm in each year and about 12% of new born babies are preterm in USA [1]. It has been recognized that term labor is the consequences of physiological condition during pregnancy, while preterm labor is the pathological activation that occurs due to multiple factors [2]. The experimental and clinical evidence suggested that preterm labor occur due to the result of decidual hemorrhage, inflammation, contraction of myometrium membrane as well as Maternal and fetal hypothalamic pituitary adrenal axis (HPA axis) activation. These multiple factors trigger parturition and also involved in the activation of myometrial cell membrane as well as enhance the activity of genital tract protease. These proteases cause cervical changes and preterm premature rupture of membranes (PPROM). Moreover, myometrium can be activated by increased production of oxytocin hormone and prostaglandin. Activation of hormones can be triggered by increase stimulation of fetal hypothalamic pituitary adrenal (HPA) axis and contraction of uterus. Cortisol hormone is also implicated to enhance the level of prostaglandin in decidua and fetal membrane via down regulation of PGDH (15-hydroxy prostaglandin dehydrogenase) and up-regulation of PGHS (Prostaglandin endoperoxidase H synthase) [3].

All newborn babies represent the low levels of RBCs in the first week of life that shows the physiological variations in the extra-uterine environments [4]. There is faster reduction of hemoglobin in preterm babies and maximum value of difference has found in preterm and full-term newborns, that state is known as anemia of prematurity (AOP). However, there are multiple factors which are implicated in anemia of prematurity including increase number of blood sample collection for laboratory test, reduction in life time of neonatal erythrocytes, growth of somatic cells in children, decline transfusion of fetoplacental during baby birth as well as disruption in the secretion of erythropoietin (EPO) [5]. According to various studies, insufficient production of serum erythropoietin has found in preterm anemic

babies [6]. For the treatment of AOP (Anemia of prematurity), blood transfusion is most widely used method in which approximately 80% of newborns are recovered. However, the procedure of blood transfusion is not totally safe and may carry out various complications including hypercalcemia, hemolysis and cardio-pulmonary diseases [7]. Moreover, the anemia of prematurity is the difference between the levels of hemoglobin (HB) and haematocrit [8-9].

Oxidative stress is the result of imbalances between pro-oxidants (Reducing agents) and anti-oxidants. These anti-oxidants are enzymatic and non-enzymatic that involved in the elimination of reactive oxygen species and free radicals. The result of oxidative stress on the fetal environment triggers the multifarious array of genes which are implicated in coagulation, inflammation, signal transduction, fibrinolysis and cell cycle [10]. It has also established that reactive oxygen species (ROS) have crucial role in fertilization and developing embryos [11]. However in the presence of adequate amount of anti-oxidants, free radicals are constantly synthesized in biological environment and are necessary for fetal growth and cell metabolism [12]. Various mechanisms such as inflammation, hyperoxia, hypoxia, damaging of endothelial lining, arachidonic acid cascade and Fenton reaction are all associated with the synthesis of reactive products. Preterm infants are more prone to free radicals that lead to immaturity and infants are commonly exposed to hyperoxia and oxygen therapy. Furthermore, the preterm infants have increased vulnerability to inflammation and infection that raise oxidative stress [13]. The increase oxidative stress in hypoxic neonates and fetuses has determined by the measuring the level of lipid peroxidation, Malondialdehyde (MDA), Advance oxidative protein products (AOPPs), serum hydroperoxides, serum NPBI (Non-protein bound iron) and increased serum Isoprostanes. Meanwhile, decrease levels of serum anti-oxidants has also found in red cells of preterm anemic babies [14].

MATERIALS AND METHODS

The aim of this study was to evaluate the oxidative stress and inflammatory markers in preterm infants suffering from anemia, 100 samples of preterm infants were added and 100 samples of full-term infants were taken as a control. All the experiments were conducted in accordance with the standards approved by Research Ethical Committee of Institute of Molecular Biology and Biotechnology; The University of Lahore.

From anti-cubital vein of each participant, five ml of blood samples were taken. Within one hour of collection, centrifugation of samples was done and serum was stored at -70°C until assayed.

BIOCHEMICAL ANALYSIS

Lipid peroxidation in the form of MDA was determined calorimetrically by using the method of Ohkawa *et al* [15]. In each test tube 200µl of sample was taken and 200µl of SDS (8.1%) was added. Then 1.5ml of acetic acid (20%) and TBA (0.8%) were mixed in each test tube and incubated for 60 min. 4ml of butanol was mixed after cooling of test tubes and centrifuged at 3000rpm for 10 min. by using the method of Moron *et al* [16]. GSH was estimated by Ellman's reagents (DTNB) reacts with glutathione, a chromophore TNB and oxidized glutathione are formed and absorbance was taken at 412nm. Levels of Catalase in the blood sample were measured spectrophotometrically by using the method of Aebi [17]. Glutathione peroxidase was estimated spectrophotometrically by using buffer/enzyme reagent [18]. Superoxide dismutase was measured by using the method of Kakkar [19]. Levels of IL-6 and TNF-α was estimated by using commercially available Elisa Kit.

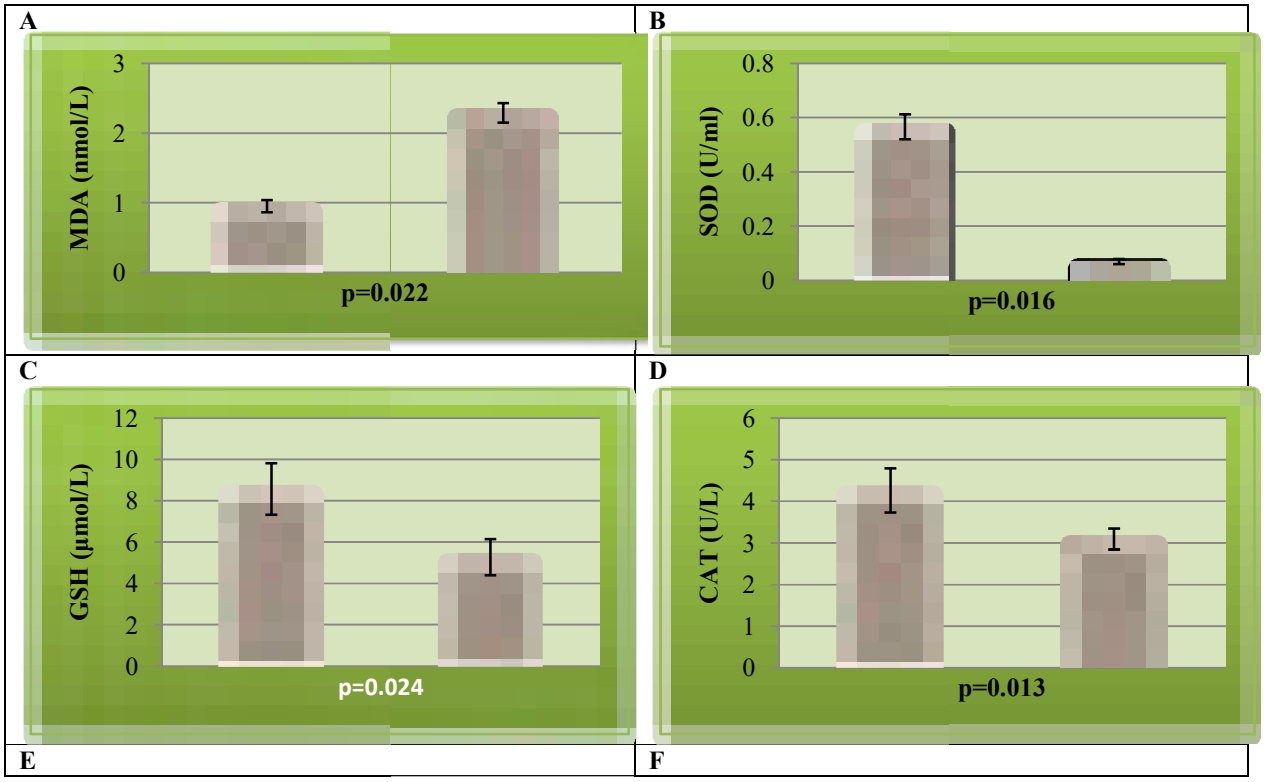
RESULTS

The data presented in figure 1 indicates the various circulating biomarkers profile that is evaluated in preterm infants suffering from anemia. While measuring the biomarkers of oxidative stress as shown in figure 1 (A) indicates the increase level of MDA in preterm anemic infants (2.29±0.95 nmol/ml) as compared to full-term infants (0.95±0.019 nmol/ml). According to figure 1 (G), serum NO level was remarkably increased in preterm anemic infants (42.26±3.88 µmol/L) in contrary to full-term infants (21.05±3.99 µmol/L). As describe in figure 1 (B), the levels of anti-oxidants were determined in which the levels of SOD in preterm infants were significantly lower as compared to full-term infants (0.069±0.005 Vs 0.566±0.016 IU/ml). As illustrated in figure 1 (D), the serum levels of CAT in preterm infants was significantly decreases (3.09±1.09 IU/L) in contrary to full-term infants (4.26±1.26 IU/L). The figure 1 (C) signifies that GSH levels in preterm anemic infants was distinctly reduced (5.26±1.55 µmol/L) in comparison with full-term healthy infants (8.56±1.25 µmol/L) correspondingly. As shown in figure 1 (H), the levels of serum GPx in preterm infants significantly decrease (6.25±1.58 µmol/L) as compared to full-term infants (7.29±1.14 µmol/L). On the

other hand, according to the figure 1 (H) the serum TNF-α level was remarkably higher in preterm infants as compared to full-term healthy infants (43.26±4.26 vs 21.08±3.88 pg/ml). Meanwhile, the figure 1 (G) illustrate the levels of IL-6 which was also increased in preterm anemic infants (7.16±2.16 pg/ml) as contrary to full-term infants (5.69±1.55 pg/ml).

DISCUSSION

Preterm birth also called as premature birth, it is the birth of newborn which initiates before 37 weeks of gestation. The etiology of preterm birth is still unknown but the major risk factors include preeclampsia, poor nutrition, gestational diabetes, vaginal infections, smoking, psychological stress, placental abruption, multiple pregnancies, hormonal imbalances and anemia that lead to low fetal iron storage in preterm and full term infants [20]. The experimental and clinical evidence suggest that preterm birth occurs due to the activation of fetal and maternal HPA (Hypothalamic pituitary axis), decidual hemorrhage, systemic inflammation and myometrium contraction. These biological events are responsible to enhance the activity of pre-term premature rupture of membrane (PPROM) and cause cervical changes.



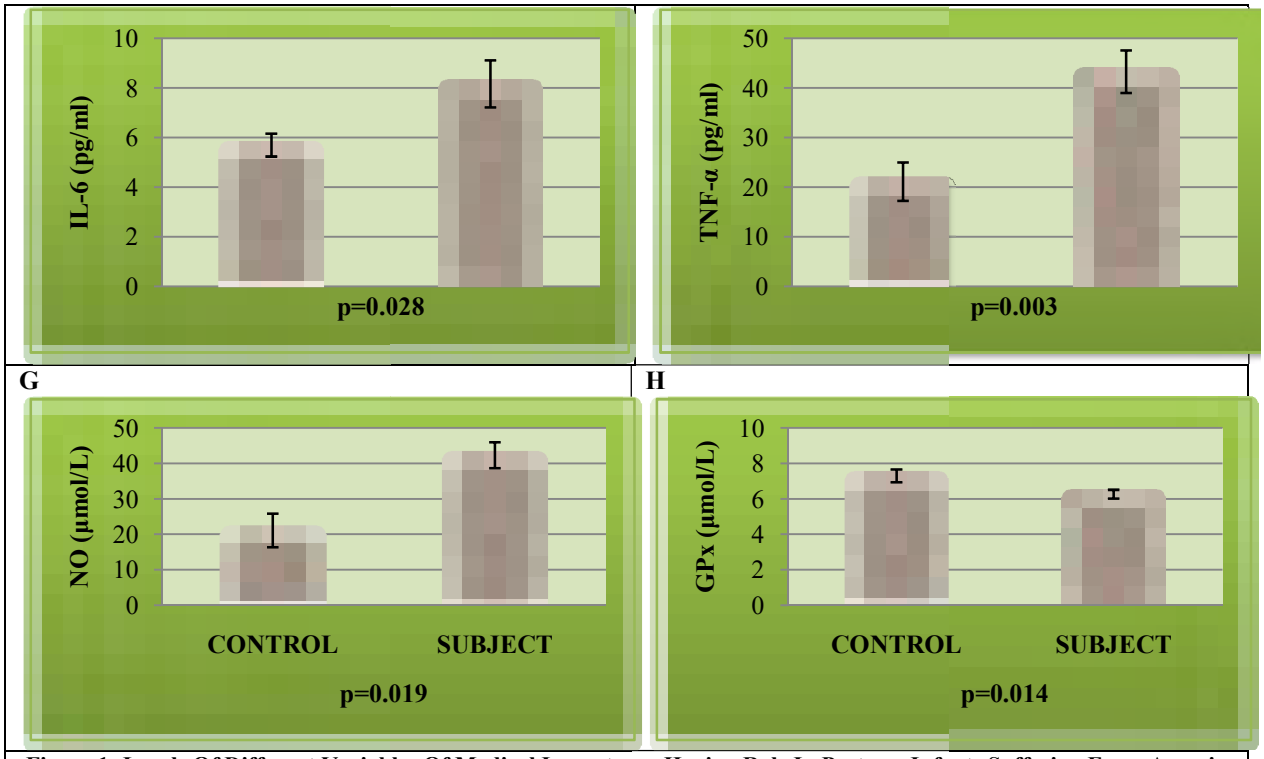


Figure 1: Levels Of Different Variables Of Medical Importance Having Role In Preterm Infants Suffering From Anemia

Preterm delivery is also due to the result of elevated uterine contractility and stimulation as well as the activation of myometrium wall. The myometrium membrane is activated by raised levels of oxytocin hormone and prostaglandin. These hormones are responsible to enhance the contraction of myometrium and cause preterm delivery of infants. Moreover, preterm birth has been associated with several morbidities in which anemia are important one. Preterm infants are suffering from anemia due to increased demand of iron and loss of blood because of physiological decline of hemoglobin levels and phlebotomy [21]. Decline of total body iron in preterm infant's increases with the decrease of gestational age and it became aggravated in premature infants [22]. During the condition of iron deficiency anemia in preterm infants, the existence of red blood cells is decreased. However, the decrease level of iron is responsible to enhance the potential of oxidative insults that cause lipid peroxidation and decline the concentrations of anti-oxidants enzymes including Glutathione peroxidase, Catalase and superoxide dismutase. Anti-oxidants defense mechanism is not accelerate in preterm infants due to oxidative insults and trigger tissue injury by the synthesis of ROS (Reactive oxygen species) and free radicals [23]. Moreover, antioxidants enzymes contribute in the redox reaction of molecules

that are involved in various cellular processes to regulate placental, postnatal and fetal growth [24]. In the current study, there are decreased levels of enzymatic antioxidants in preterm anemic infants as compared to full-term infants. The research carried out by Thibeault, 2000 [25] also reviewed the similar trend i.e., decreased enzymes and increased production of reactive oxygen species (ROS). One of the important enzymatic anti-oxidant is SOD (Superoxide dismutase), that is expressed in peroxisomes, cytoplasm as well as mitochondria [25]. It is essential to catalyze dismutation of superoxide radical into hydrogen peroxide. Similarly, catalase in peroxisomes and glutathione peroxidase in mitochondria are involved in the conversion of hydrogen peroxide into water and oxygen.

Furthermore, as the oxidative insult increases it contributes in the progression and development of anemia, the appropriate mechanism by which it occurs is still under investigation. Malondialdehyde (MDA) that is the end product of lipid peroxidation and can be formed by decomposition of arachidonic acid and polyunsaturated fatty acid (PUFA) [26]. The current study signified the levels of MDA in the serum sample of preterm anemic babies compared with control subjects. It revealed increased MDA in preterm infants as compared to healthy individuals. These findings remained similar to the study performed by Cecile *et al* [14]. Oxidative stress is responsible in liberation of various inflammatory cytokines by different mechanisms. There are elevated levels of cytokines such as TNF-α and IL-6 in the present study which shows their role in inflammation and infection in such infants. Increased cytokines along with increased lipid peroxidation is measured by the levels of MDA in preterm anemic infants (IL-6 Vs. MDA, r= 0.738*** and TNF-α Vs. MDA, r= 0.601**).

CONCLUSION

Pregnant women with their developing preterm infants and fetus are highly prone to oxidative insults because they are more exposed to inflammatory/infectious conditions, maternal hypertension, and diabetes during pregnancy and anemia due to low iron intake. All of these factors are responsible to enhance the production of ROS and free radicals. It has also reported that during pregnancy and in preterm infants, there are decreased concentrations of antioxidants which are unable to balance oxidative stress. Preterm infants suffer in anemia due increased demand of iron and

the loss of blood. In the condition of iron deficiency anemia in preterm infants, the survival of RBCs is diminished. The decrease level of iron is responsible to enhance the potential of oxidative stress that cause lipid peroxidation, decline the concentration of antioxidants and secrete inflammatory cytokines.

ACKNOWLEDGEMENTS

The authors are highly thankful to Lab-313, Institute of Molecular Biology and Biotechnology, The University of Lahore-Pakistan to provide the innovation and financial support for the project.

CONFLICT OF INTEREST

Author declares no conflict of interest.

REFERENCES

[1] Martin JA, Hamilton BE, Sutton PD. Births: final data for 2007. National vital statistics reports: from the Centers for Disease Control and Prevention, National Center for Health Statistics. National Vital Statistics System, (2010); 58: 1-85.

[2] Ivanisevic M, Djelmis J, Bukovic D. Review on Prostaglandin and Oxytocin Activity in Preterm Labor, Coll Antropol. (2001); 2(2): 687-694.

[3] Challis JR, Smith SK. Biology of the Neonate, Intern J Gynaecol Obstet, (2001); 79:163.

[4] Bechensteen AG, Haga P, Halvorsen S, Whiterlaw A, Liestøl K, Lindermann R, et al. Erythropoietin, protein, and iron supplementation and the prevention of anaemia of prematurity. Arch Dis Child, (1993); 69: 19-23.

[5] Widness JA, Seward VJ, Kromer IJ, Burmeister LF, Bell EF, Strauss RG, Changing patterns of red blood cell transfusion in very low birth weight infants. J Pediatr, (1996); 129: 680-7.

[6] Halperin DS. Use of recombinant erythropoietin in treatment of the anemia of prematurity. Am J Pediatr Hematol Oncol, (1991); 13: 351-63.

[7] Juul SE. Erythropoietin in the neonate. Curr Probl Pediatr, (1999); 29: 133-49.

[8] Miller Y, Bachowski G, Benjamin R. Practice guidelines for blood transfusion. Americn Red Cross, (2007); 46: 279-85.

[9] Gibson BE, Todd A, Roberts I, Pamphilon D, Rodeck C, Bolton-Maggs P, et al. Transfusion guidelines for neonates and older children. Br J Haematol. 2004; 124(4): 433-53.

[10] Wagenaar GT, Horst SA, van Gastelen MA, Leijser LM, Mauad T, van der Velden PA, et al. Gene expression profile and histopathology of experimental bronchopulmonary dysplasia induced by

prolonged oxidative stress. Free Radic Biol Med, (2004); 36: 782-801.

[11] Dennery PA. Role of redox in fetal development and neonatal diseases. Antioxid Redox Signal, (2004); 6: 147-53.

[12] Halliwell B. Free radicals, antioxidants and human disease: curiosity, cause or consequence? Lancet, (1994); 344:721-24.

[13] Speer CP, Silverman M. Issues relating to children born prematurely. EurRespir J Suppl, (1998); 27: 13s-16s.

[14] Cecile C, Stephane H, Delphine MB, Jean-Paul S, Jean CP. Adduct of malondialdehyde to hemoglobin: A new marker of oxidative stress that is associated with significant morbidity in preterm infants, Oxidative medicine and cellular Longevity. (2013); 8.

[15] Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical biochemistry, (1979); 95(2): 351-8.

[16] Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S transferase activities in rat lung and liver. Biochimicaet Biophysica Acta, (1979); 582(1): 67-78.

[17] Abei H. Catalase in Bergmeyer HU methods in enzymatic analysis: New York, Academic press. (1979); 3: 276-86.

[18] Abei HE, Bergmeyer HU. In methods of enzymatic analysis. Verlag Chemicals, (1983); 3273-86.

[19] Kakkar PB, Das P, Viswanathan PN. A modified spectrophotometer assay of superoxide dismutase. Ind J Biochem Bio, (1984); 3: 273-86.

[20] Lockwood CJ. The diagnosis of preterm labor and the prediction of preterm delivery. ClinObstet Gynecol, (1995); 38: 675-87.

[21] Armony-Sivan R, Eidelman AI, Lanir A, Sredni D, Yehuda S. Iron status and neurobehavioral development of premature infants. J Perinatol, (2004); 24(12): 757-62.

[22] Tran PV, Fretham SJ, Carlson ES, Georgieff MK. Long-term reduction of hippocampal brain-derived neurotrophic factor activity after fetal-neonatal iron deficiency in adult rats. Pediator Res, (2009); 65(5): 493-498.

[23] Baba L, McGrath JM. Oxygen free radicals: effects in the newborn period. Adv Neonatal Care, (2008); 8(5): 256-264.

[24] Dennery PA, Role of redox in fetal development and neonatal diseases.

Antioxid Redox Signal, (2004); 6: 147-153.

[25] Thibeault DW. The precarious antioxidant defenses of the preterm infant. Am J Perinatol. (2000); 4: 167-181.

[26] Rasool M, Malik A, Basit Ashraf MA, Parveen G, Iqbal S, Ali I, et al. Evaluation of Matrix Metalloproteinases, Cytokines and Their Potential Role in the Development of Ovarian Cancer. PLoS ONE 2016; 11(11).