Aging Influence Mitochondrial Dysfunction and Oxidative Stress through Check Some Proinflammatory Cytokines Levels and Oxidant Status in Older Adults: an Observational Study

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Abstract

The experiment design is that of a cohort study within the setting of a medium private laboratory with participants being common people (healthy individuals of 20–99 years=108; mean age 55 years and 71.3% man) were classified into six age groups. Oxygen saturation (SpO2), pulse rate, systolic blood pressure (CSBP) and diastolic blood pressure were assessed. Blood sera (once) was obtained for proinflammatory cytokines levels (including c-reactive protein (CRP), insulin growth factor-1 (IGF-1), apolipoprotein E (apoE), interleukin 6 (IL-6), 8-hydroxy-2'–deoxyguanosine (8-OHdG) and tumor necrosis factor-α (TNF-α)) and oxidant status (including malondialdehyde (MDA), superoxide dismutase (SOD) and total antioxidant capacity (T-AOC) analysis. The mean calculated of the whole participants were equal for proinflammatory cytokines levels: CRP 3.24 mg/dL, IGF-1 54.03 ng/mL, apoE 488.29 mg/mL, IL-6 65.464 pg/mL, 8-OHdG 1.34 ng/mL and TNF-α 105.63 pg/mL; and oxidant statue: MDA 0.59 ng/mL, SOD 3.48 U/mL and T-AOC 1.21 mmol/L, receptively. Our findings highlighted that survey of mitochondrial dysfunction and oxidative stress through check some proinflammatory cytokines levels and oxidant status of aging in older adults can be applied to build a global frailty index as a tool to quantify aging in preclinical experiments.

Keywords: *Physiological aging, Proinflammatory cytokines, Oxidant status, Adults, Iraq*

Introduction

The world population continues to grow older rapidly, mostly because of declining fertility and increasing longevity [49] . For this reason, aging research has experienced an unprecedented advance over recent years, particularly with the discovery that the rate of aging is controlled, at least to some extent, by biochemical processes and genetic pathways conserved in evolution [29].

Aging is a process of the progressive functional decline of various physiological functions in various organs and tissues with time, which lead to disability, dependence, morbidity, and mortality [9] . This reduction manifests as a decreased physiological reserve in response to a time-dependent failure of complex molecular mechanisms and stress (termed homeostenosis) that cumulatively create disorder [23].

Given the intricacy of the ageing process in organism, the underlying exact mechanisms are still not good understood. In current literature, free radical theory of aging is the most intriguing among the various mechanisms that are postulated to participate in the course of aging. And, mitochondria are important determinants of cellular homeostasis and longevity since they are the main producers of cellular ATP and play a vital role in regulation of apoptotic death pathways in many tissues [51]. And, based on free radical theory of aging mitochondria are the origin source of intracellular reactive oxygen species (ROS, which are not readily

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removed) in normal human aging. ROS accumulate as side products of the electron transport chain, causing mitochondrial dysfunction [30].

In the past decade, efforts to develop an aging metric based on genetic profiles, epigenetic changes and telomere length have met with limited success [34]. Thus, inflammatory cytokines and oxidant status proportion in the blood may be practical to monitoring those at risk of cognitive and functional decline in older adults aging. The main aim this experiment a set of unifying hallmarks of free radical theory of aging that has been defined providing an outline for understanding the process of mitochondrial ageing and oxidative at older adults function through check some proinflammatory cytokines levels and oxidant status.

Materials and Method

Experimental design

The experiment design is that of a cohort study within the setting of a medium private laboratory with participants being common people (healthy individuals of 20–99 years=108; mean age 55 years and 71.3% man) were classified into six groups according to age of participants (years): I) 20-39 (n=15); II) 40-49 (n=24); III) 50-59 (n=23); IV) 60-69 (n=22); V) 70-79 (n=13) and VI) 80-99 (n=11). All samples were obtained from Sulaimani Nursing House and New Medical Center (Private Laboratory) from December-2016 to April-2017. Experimental protocols were approved by the Ethics Review Committee of Medicine, Sulaimani Nursing House, Iraq (approval no: 0529.1.75/3).

Data collection

Reagents and equipment were purchased from Roche (Germany). Before blood sampling, operators medical were took some clinical examination from participants including oxygen saturation variability (as described previously by [1]), pulse rate, systolic blood pressure (SBP) and diastolic blood pressure (DBP) by automated Watch Allyn sphygmomanometer (Germany). Fasting blood samples (once) were obtained of all participants into vacuum tubes (non-heparinized) for the assay of serum. The serum was separated by centrifugation (15,000×g for 10 min), kept at –80 °C and assessed by Human ELISAs kit, as recommended by the manufacturer.

Sera proinflammatory cytokines and oxidant measurements

The sample characteristics of proinflammatory cytokines and oxidant statue kits are shown in Table 1. Blood sera was obtained for assay proinflammatory cytokines levels (including c-reactive protein (CRP), insulin growth factor-1 (IGF-1), apolipoprotein E (apoE), IL-6, 8-hydroxy-2'–deoxyguanosine (8-OHdG), and tumor necrosis factor-α TNF-α) and oxidant statue (including MDA, SOD, and total antioxidant capacity (T-AOC)) analysis.

Table 1: Sample characteristics of proinflammatory cytokines and antioxidant kits

Parameters	Cat number	Sensitivity	Range	Company
CRP (mg/L)	cat# 20764930322	<5.0	1.0-200	Roche Cobas Integra 400 plus
IGF-1 (ng/mL)	cat# EELH0086	<0.94	1.56–100	Elabscience Biotechnology Inc.
apoE (ng/mL)	cat# EELH0470	<14.06	23.44-1500	Elabscience Biotechnology Inc.
IL-6 (pg/mL)	cat# EELH0102	<4.69	7.81-500	Elabscience Biotechnology Inc.
8-OHdG (ng/mL)	cat# EEL0028	<0.938	1.563-100	Elabscience Biotechnology Inc.
TNF-α (pg/mL)	cat# EELH0109	<4.69	7.81-500	Elabscience Biotechnology Inc.
MDA (ng/mL)	cat# EELH0060	< 18.75	31.25–2000	Elabscience Biotechnology Inc.
SOD (U/mL)	cat# BC0020	0.2	0.2 -14.4	Elabscience Biotechnology Inc.
T-AOC (mmol/L)	cat# BC0219	0.047	0.047-1.5	Elabscience Biotechnology Inc.

CRP: c-reactive protein; IGF-1: insulin growth factor-1; apoE: apolipoprotein E; IL-6: interleukin 6; 8-OHdG: 8-hydroxy-2'–deoxyguanosine; TNF-α: tumor necrosis factor-α; MDA: malondialdehyde; SOD: super oxidase dismutase; T-AOC: total antioxidant capacity

Statistical Analysis

The results were analyzed using the SPSS 22 software. ANOVA and post-hoc LSD test was used to test differences between treatments. The results were expressed as the mean ± standard deviation (SD). Differences with values of $p<0.05$ were considered statistically significant.

Results

No participant recruited chose to withdraw from the experiment then all participants were considered for analysis. The descriptive statistics presented in Table 1, show that the highest (97.4±0.99) and least (95.45±2.77) mean of oxygen saturation (SpO2) in groups of 20-39 and 80-99 years, respectively. And, the only significant differences were detected between the groups 70-79 and 80-99 years ($p<0.05$). The analysis revealed that significant differences for pulse rate (p=0.037) and systolic blood pressure (p=0.005) were detected between the means of the age groups, but the pattern was not consistent. The mean pulse rate of the whole participants was 78.06 beat/minute. Higher means were detected in the young and the old age groups, and the lowest were observed in the 50-69 years group. The highest (136.82±11.89) and least (119.71±13.2) mean of systolic blood pressure (mmHg) were shown in groups of 80-99 and 40-49 years, respectively. The diastolic blood pressure of the whole sample was 84 mmHg. The highest mean (107.83±12.5) diastolic blood pressure (mmHg) was in the age group 50-59 years, but the difference was not significant (p=0.482).

Table 2: Mean ±SD of SpO2, pulse rate, SBP and DBP, stratified by age

Parameter	20-39	40-49	50-59	60-69	70-79	80-99
SpO2 (%)	97.40±0.99a	97.29±0.91a	97.00±0.74a	97.18±1.22a	96.00±1.78b	95.45±2.77b
Pulse rate (%)	83.47±10.41a	80.08±14.68ab	74.78±9.86 ab	72.41±10.62b	81.31±13.58 ab	80.55±8.69 ab
SBP (mmHg)	126.7±11.75ab	119.7±13.20a	135.7±18.61b	134.8±15.47b	132.5±21.55b	136.8±11.89b
DBP (mmHg)	77.33±8.84	76.54±7.38	81.52±11.02	77.95±10.87	77.69±10.53	79.09±7.35

SpO2: oxygen saturation; SBP: systolic blood pressure; DBP: diastolic blood pressure

The same letters mean non-significant difference, while the different letters mean significant difference at p<0.05

Table 3 and 4 provides a summary of the outcomes for sera levels of proinflammatory cytokines and oxidant statue by treatment group and study time points. The mean calculated of the whole participants were equal for proinflammatory cytokines levels: CRP 3.24 mg/L, IGF-1 54.03 ng/mL, apoE 488.29 mg/mL, IL-6 65.46 pg/mL, 8-OHdG 1.34 ng/mL and TNF-α 105.63 pg/mL; and oxidant statue: MDA 0.59 ng/mL, SOD 3.48 U/mL and T-AOC 1.21 mmol/L, receptively. Several parameters significantly differed between age groups. The blood sera analysis in all age groups revealed that the levels of some proinflammatory cytokines (except IGF-1) and oxidant statue (except MDA) adults in middle to late adulthood significantly decreased (p<0.001). The kits analysis revealed that the levels of IGF-1 (chemiluminescent/immunometric assay) and MDA (colorimetric/fluorometric assay) adults in middle to late adulthood decreased and increased, receptively (p<0.001). LSD test showed that mean of IGF-1 the significant difference between each of two groups except for the difference between groups of 70-79 and 80-99 years (p=0.244). In contrary, mean of MDA the significant difference between each of two groups except for the difference between groups of 20-39 and 40-49 years (p=0.975).

Table 3: Serum levels of CRP, IGF-1, APO-E, IL-6, 8-OHdG and TNF-α, stratified by age

Parameter	20-39	40-49	50-59	60-69	70-79	80-99
CRP (mg/dL)	2.44±0.98[a]	2.82±1.84[ab]	3.14±1.60 [ab]	3.11±1.25[ab]	3.74±1.42[b]	5.06±1.41[c]
IGF-1 (ng/mL)	78.36±7.14[a]	71.35±6.44[b]	60.06±4.75[c]	51.56±2.97[d]	40.23±4.37[e]	38.15±7.70[e]
APO-E (mg/mL)	251.9±74.48[a]	383.0±66.84[b]	409.59±80.18[b]	573.4±102.6[c]	668.1±87.70[d]	821.9±77.68[e]
IL-6 (pg/mL)	46.73±4.07[a]	51.24±3.87[b]	60.69±6.62[c]	70.51±5.59[d]	87.90±6.32[e]	95.45±4.62[f]
8-OHdG (ng/mL)	0.43±0.08[a]	0.89±0.08 [b]	1.03±0.07[c]	1.40±0.08[d]	1.88±0.08[e]	2.38±0.10 [a]
TNF-α (pg/mL)	59.92±7.44[a]	75.43±8.78[b]	91.33±8.66[c]	121.13±15.1[d]	157.5±8.85[e]	171.5±17.42[f]

CRP: c-reactive protein; IGF-1: insulin growth factor-1; apoE: apolipoprotein E; IL-6: interleukin 6; 8-OHdG: 8-hydroxy-2'–deoxyguanosine; TNF-α: tumor necrosis factor-α.

Note: Results expressed as Mean ±SD. The same letters mean non-significant difference, while the different letters mean significant difference at $p<0.05$

Table 4: Serum levels of MDA, SOD and T-AOC, stratified by age

Parameter	20-39	40-49	50-59	60-69	70-79	80-99
MDA (ng/mL)	0.45±0.09a	0.45±0.06a	0.55±0.04b	0.64±0.06c	0.73±0.10d	0.91±0.07e
SOD (U/mL)	3.95±0.68 a	3.99±0.42 a	3.49±0.63 b	3.18±0.62 b	3.18±0.46 b	2.64±0.23c
T-AOC (mmol/L)	1.42±0.32a	1.41±0.14 a	1.27±0.11b	1.16±0.10b	1.04±0.10c	0.67±0.13d

MDA: malondialdehyde; SOD: super oxidase dismutase; T-AOC: total antioxidant capacity

Note: Results expressed as Mean ±SD. The same letters mean non-significant difference, while the different letters mean significant difference at $p<0.05$

Discussion

Pulse oximetry is a method used to quantify SpO2 non-invasively. And, it is a technique regularly utilized clinically whether that be in concentrated consideration, in medical procedure, or in some out-patient clinics [21]. The results current experiment showed no substantial variance between groups in association with SpO2, only a slight decreasing was found in group of 80-90 years age, and it's in agreement with the study of [1] in which the SpO2 showed no substantial variance in groups under 35 ages and above 50 ages. Other previous studies indicated that there were a number of ways in which free radicals are formed but their most abundant source are the mitochondria (which uses some 90% of the O2 used by the human body) where oxygen is reduced in sequential steps to produce water [14]. CRP functions as a marker of systemic inflammation and is one of the top studied inflammatory biomarkers in coronary arterial disease [22]. According to the current edition of Harrison's Principles of Internal Medicine (2012) the normal range of CRP 0.2–3.0 mg/L, while the presently obtained results showed that adults in middle to late adulthood showed higher serum CRP levels 3.0-5.0 mg/L (Table 3). Importantly, the population differences should also be taken into multiple factors including genetics, environmental conditions, developmental programming determine maximal organ function [26], diet and polymorphism [57] which varies significantly between individuals.

In summary, [31] described that IGF-1 the cross road of the nutritional, inflammatory and hormonal pathways to frailty. And, targeted deletion of specific genes has demonstrated that multiple components of the IGF-1/insulin signaling pathway play a role in the aging process spanning from nematodes to rodents [3]. This hormone endorses cell survival, prevents apoptosis, and motivates neurogenesis in the hippocampus that affected early in Alzheimer disease [27]. The results of our investigation showed that the minimum IGF-1 (ng/mL) mean (38.15) was observed in group 80-99 years age, while the maximum mean (78.36) was displayed in group 20-39 years age (Table 3).

Previous studies indicated that apoE is a useful player in studies of longevity and age-related diseases, such as inflammatory status and atherosclerosis that are known risk factors for functional decline and early mortality [5]. Moreover, it is possible that apoE may also play a role in other pathological conditions including, for example, cancer, rheumatoid arthritis and macular degeneration, but the exact biological mechanisms underlying these observations are poorly understood until now [5] . Our study observed the least (251.97±74.48) and highest (821.98±77.68) mean of apoE (ng/mL) were shown in groups of 20-39 and 80-99 years, respectively (Table 3).

Measuring biomarker signatures of inflammation, such as IL-6, TNF-a, and IL-1β that change with aging may be an effective way to assess for inflammaging in older adults and thus risk for inflammatory diseases with high morbidity and mortality rates [46]. The results observed in the present study that extreme mean of IL-6 (95.45 pg/mL) and TNF-α (171.46 pg/mL) in group 80-99 years, and the minimum mean IL-6 (46.73 pg/mL) and TNF-α (59.92 pg/mL) in group 20-39 years age (Table 3).

Conclusion

This study might provide a therapeutic target for aging and age-related disease. On the other hand, ‘‘check some proinflammatory cytokines levels and oxidant status’’, can be itself considered a hallmark of ageing.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under Sulaimany Technical Institute, Sulaimany Polytechnic University, Sulaimany, Iraq and all experiments were carried out in accordance with approved guidelines.

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