

The hidden impact of modern life on D-dimer level: an experimental study

Sama Attiyah¹

¹ Associate professor of immunology and cardiology, University of Jeddah, Saudi Arabia.

Abstract. Social and life habits are among the underline cause of several diseases especially coronary heart disease and stroke. Because high fat diet, sleep deficit, and anxiety are among diseases hidden elements, the present research aimed to evaluate the association between those factors and plasma D- dimer production level. Three groups in addition to control cluster of male swiss albino mice were investigated (n=56). Each cluster 16 mice and had different experimental condition for four weeks. High fat diet, electrostatic noise, and deficit of sleep were applied seperately for each group under investigation. Blood was collected and plasma D-dimer level was assessed at the end of each week. The student's t-test was used for statistical analysis. Gradual substantial increase of plasma D-dimer amount were identified with all conditions under investigate. Anxiety and lack of sleep provide signeficant plasma D-dimer amount $p < 0.001$ and $p < 0.001$ respectively. Our results explicitly implicate that prolonged sleep defect and anxiety by noise may trigger a direct stress response which have a significant connection to the raise of D-dimer production level, that known as a major source of thrombosis risk. Regulating these elements in an individual's life is essential to mitigate the proliferation of many inflammatory diseases, including atherosclerosis, stroke, and mortality.

1 Introduction

Diseases include stroke, heart failure, obesity, high blood pressuer, kidney and liver disease demonstrate increased dramatically nowadays [1]. Modern lifestyles play majour role in people health condition which draw the baseline of diseases strength and spread. Atherosclerosis is one of the most common type of heart disease that causes death in both genders all over the world [2]. Several acquired habits of moderen lifestyle taht irritate and negatively affect human immune system should be controlled in all societies especially among new generation [3, 4]. Human diet has become determined mainly by carbohydrates and fatty foods such as burgers and donut [5]. In addition to the lack of movement and the increase of stress in individuals' lives, and the expanding of lack of sleep incident all showed mental health problems [4, 6]. Each habit led to specific health problem: high fat diet cause diabetic, heart and brain diseases, shortage of sleep and the increase of anxiety both influence human underline inflammation factors include D- dimer, IL-1, IL-2, IL-6, C-reactive protein (CRP), TNF- α , IFN- γ [6, 7, 8].

Plasma D-dimer is a crucial marker indicate to inflammation process especially for atherosclerosis, depression, high blood pressure, and diabetic [6, 9]. In 1987 Hillyard and his

group demonstrated D-dimer as thrombosis important marker [10]. The continued use of plasma D-dimers test from year 1987 up to 2024 proves the importance of this biomarker in the medical field [11, 12]. D-dimer clinical test is a method able to identify the underline illness process and diagnosis which is completed in minutes and at a cheap price [13] It is applied normally in all thrombosis incident types as one of primary plasma test which successfully detect serious illness [14, 15]. Risk factors that contribute to the anxiety, sleep deficit, and consuming unhealthy food involved in high blood pressure ($\geq 140/90$ mmHg), low levels of high-density lipoprotein (HDL) in combination with high levels of low-density lipoprotein (LDL), all are considered as crucial factors in the development of main illness [5, 16]. Other important factors include age, previous family history, smoking and gender are involved as well. Several other types of pollution appeared in modern life negatively affect human health, for instance: noise (e.g. industrial machines and traffics), environment (e.g. pesticides), light (e.g. miss use of artificial light), and air pollution (e.g. factories and forest) [17, 18].

The development of chronic diseases has escalated among young people, in addition to the severity and danger of diseases among elderly due to the uncontrollable lifestyle conditions and the spread of new technologies (Almeida et al., 2011; Anderson et al., 2013; Zhou et al., 2024). Despite the great development in industries and in modern technology used in the medical field, the world suffers from high rate of mortality that appeared to be as results to the nature of modern life, which is difficult to control [3, 4]. Importantly, in 2018 a clinical trial published by Simes and his group clarified that plasma D-dimer level is a crucial risk factor induce mortality by four time among other reasons, and by 13.6 time for venous thromboembolism illness [19].

The existing experimental analysis aimed to estimate the induction of plasma D-dimer level in mice model under investigation conditions (high fat diet, sleep deficit, anxiety), and indicate to which of condition under investigation more contribute to thrombosis and atherosclerosis.

2 Martial and methods

2.1 In-vitro Study

Prior to experiment, mice were purchased from the European Country Farms in Egypt and the health check assessment was completed. In vivo study carried out using fifty six male swiss albino mice each weight around 30 gm with age between 6 to 8 weeks old. Mice were housed in temperature-controlled conditions contain pathogen-free setting cages, 60% humidity, and a temperature of 21 C°. The recommendation of (Gide For Care And use Of Laboratory Animals) was followed to fed and handle mice (water ad-libitum and standard laboratory diet containing 24% protein, 4% fat, 4-5% fiber). Mice divided in four groups each group stayed under different life condition for four weeks. High fat diet with high density (60 kcal% fat diet) was the nutrient type for the first group (n=16). Sleep deficit condition was achieved using the slowly rotating wheel (1.0 r.p.m) for 20 hours/day for four weeks (second group, n=16). Sleep deficit condition was achieved using the slowly rotating wheel to interfere mice sleep. The wheel cages is a good method that keep the spin condition constant for all experiment period (rotation 1.0/minute, speed 1.0 r.p.m). The rotating wheel applied 20 hours/day for four weeks (second group, n=16). Anxiety was obtained using a noise generator linked to electrostatic speaker and microphone which placed into cages and output for 12 hours/ day for the experiment period (third group, n=16). Last group is the control (n=8) that went through normal condition, standard rodent maintenance diet with

no especial condition. The assessment of plasma D-dimer amount was carried out using four mice at the end of each week.

2.2 Chemicals and tools

For plasma D-dimer quantification purposes: blood sample in EDTA tubes were centrifuged at 3000 rpm at 2~8°C for 15 min and supernatant was stored at 20°C. The assessment of D-dimer level in plasma was identified using 96-well plates of ELISA assay of mouse D-dimer kit [(colorimetric), Catalogue No: NBP3-08100].

2.3 Statistical analysis

Values are expressed as an average (mean) plus and minus standard deviation (\pm SD). Treatment group values were compared with the control, and the results were considered statistically significant when the probability (P) was $P < 0.05$.

3 Results

The sustained exposure to stressor factors under investigation for four weeks revealed substantial upward of D-dimer production amount compared to control group. Table 1 illustrate the statistical measures of p-values test indicate to the significant gradual buildup of plasma D-dimer ratio through experimental groups. High fat diet present the lowest increase of D-dimer level reach less than the two-fold at week four ($60 \text{ mg/l} \pm 6$, $p < 0.05$) (Figure 1). The constant extended exposure to noise stressor support plasma D-dimer dramatic raise to be more than two-folld above first week amount ($80 \text{ mg/l} \pm 3$, $p < 0.01$) (Figure 2).

As illustrated in Figure 3, sleep deprivation has been observed to significantly affect plasma D-dimer levels. Essentially, deficit of sleep showed the major boost of plasma D-dimer expression to score more than triple amount than at the initial experiment stage after four weeks of sleep deprivation ($117 \text{ mg/l} \pm 3$, $p < 0.001$) (Figure 3).

Table 1. The stimulated lvel of plasma D-dimer derived from anxiety, high fat diet and sleep dificit conditions. Data are represented as means \pm SEM, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Groups	Week 1	Week 2	Week 3	Week4
High fat diet	35 ± 2	37 ± 3	39 ± 3	60 ± 6 *
Anxiety	37 ± 3	44 ± 5	55 ± 4 *	80 ± 3 **
Sleep deficiency	38 ± 5	47 ± 3	83 ± 8 **	117 ± 3 ***

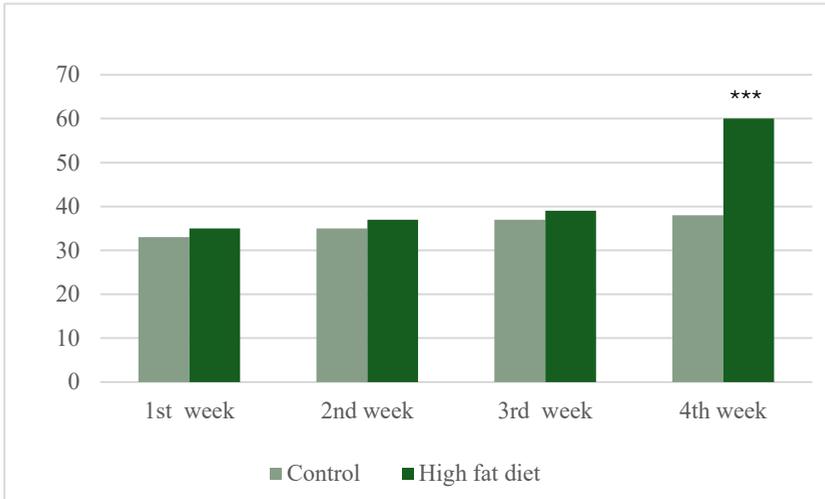


Figure 1. The association between high fat diet intake and plasma D-dimer induction level in C57BL/6 strain mice. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

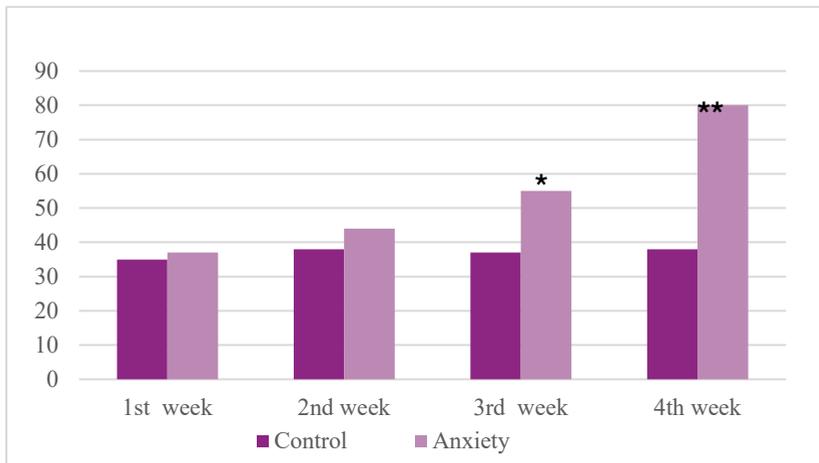


Figure 2. The consequence of anxiety (noise) on plasma D-dimer expression level in C57BL/6 strain mice. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

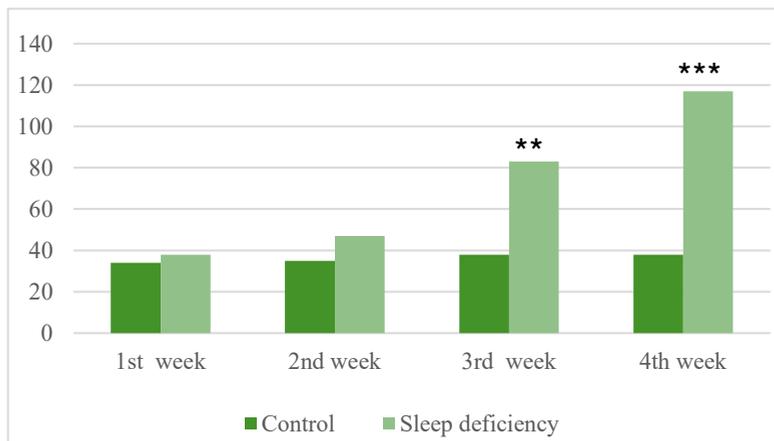


Figure 3. The consequence of sleep deficiency on plasma D-dimer expression level in C57BL/6 strain mice. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

4 Discussion

The hidden non-medical underline diseases causes are considered among the most harmful factors to human health include new lifestyle habits[4]. No visible disease symptom appears from these habits which provide good opportunity to develop illness silently. Plasma D-dimer test is a crucial method able to identify thrombosis clearly [11, 20]. D-dimer is a fragment from a fibrin protein play a role in immune response, blood clot, cardiac and brain diseases. Cardiovascular diseases (CVD) include atherosclerosis and Myocardial infarction (MI) caused by the development of arterial plaque that may derived from clinical condition or lifestyle habits such as sleepless, anxiety, smoking, or diabetes (21m 22). Borowiec and his colleagues reported taht inflammation process corelate dirictelly to the high level of plasma D-dimer production level rather than within the development of thrombosis [23]. The relation between sleepless, anxiety, and obesity with the expression of plasma D-dimer level was discovered previously [5, 21, 24]. Moreover, D-dimer plasma test has been used within COVID-19 mortality incidents as well [25]. Additionally, D-dimerhas has been identified as substantial marker for several other diseases include cancer and colerate to mortality rate [19]. Because plasma D-dimer amount is a depending prognostic marker of atherosclerosis and inflammation, the presented work shied the light on the infelucence of new life habits on the production level of this marker. Two main important findings deticted herein, 1). An expressive induce of plasma D-dimer in mice model throughout all experimental conditions indicate to the impact of different streaaful types (sleepless, high fat diet, anxiety) on underline cause of illness. 2). Diet rich of fat demonestrated a lower amount of plasma D-dimer than that induced by anxiety and sleepless conditions which may idicate to the requirement of longer period for fat intake to increase the production of D-dimer level in plasma. 3). Greatest D-dimer amount was produced by sleep dificit event showed three time increase at final experiment date. Apparently, bad moderen habits upward plasma D-dimer induction level which indirect inatiate thrombosis and other inflammation cases. The results reveal a mechanism that may explain how disrupted sleep may be related downstream to

cardiovascular risk. Our study's principal conclusion, the influence of modern living on D-dimer levels, might have significant health complications and consequences.

5 Conclusion

This study revealed that continued anxiety, sleepless, and food rich fat intake stimulate the pro-inflammatory factor amount D-dimer. Our study's main finding, the effect of contemporary life on D-dimer levels, might have serious health implications. Our results support that, in order to remain healthy in our quickly changing life, tackling sleep as a lifestyle strategy, along with a reduction of anxiety and the intake of fat food will enhance public health. A better understanding of the association between sleep loss, long-term noise exposure, as well as fat diet rich with D-dimer production levels would identifying persons of disease risk at early stage preventing danger health consequences.

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