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(The Effect Of Night Work On The Serum Levels Of The NLRP3 Inflammasome Among Bakery Workers In Northwestern Syria)

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Abstract:
Background*:*
Shift work has become a prevalent work schedule globally, known to disrupt the circadian rhythm and detrimentally impact worker health. The inflammasome NLRP3, a crucial component in disease development, responds to various stimuli, including pathogen- and damage-associated molecular patterns (PAMPs and DAMPs). Calibration of NLRP3 levels may serve as an early biomarker for health risk prediction.
Method*:*
This cross-sectional study investigated the effect of night work on serum NLRP3 Inflammasome levels among bakery workers in northwestern Syria. NLRP3 Inflammasome levels were assessed using the ELISA assay.
Results*:*
Significant differences were observed between the study and control groups, indicating that monitoring night workers' NLRP3 Inflammasome levels could facilitate early detection of health effects associated with night work.
Conclusion*:*
Tracking serum NLRP3 Inflammasome levels in night workers may offer valuable insights into the health impacts of shift work, urging the implementation of preventive measures to mitigate the risk of developing clinical disorders.

Keywords:*shift work, night work, inflammatory response, NLRP3 inflammasome, autophagy, oxidative stress.*

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I. Introduction

Night work, defined as any work system outside traditional daytime hours [1], *has become prevalent in contemporary society, with approximately 15-20% of workers in industrialized nations engaged in shift work*[2]. *The disruption it imposes on sleep-wake schedules has significant implications for health and safety* [3], *leading to its classification as a potential carcinogen by the International Agency for Research on Cancer*[4].

Shift work, including night work, has been associated with heightened levels of inflammation biomarkers [5]. *Inflammation, a multifaceted response to various stimuli, can lead to tissue damage if not properly regulated* [6]*Key to this response are pro-inflammatory cytokines, particularly tumor necrosis factor (TNF), interleukin 6 (IL-6), and interleukin 1 (IL-1), the production of which actually requires the activation of complexes such as the NLRP3 inflammasome*[7].

Activation of the NLRP3 inflammasome relies on two signals: priming by nuclear factor kappa B (NF-κB) and activation by specific triggers [8],[9]. *Excessive inflammasome activation can lead to inflammatory disorders, highlighting the importance of its regulation* [10]. *Despite its role in health and disease* [9]., *the impact of night work on NLRP3 levels in serum remains understudied* [9].

Most existing studies assessing cytokine production in relation to sleep or night work lack direct evidence of NLRP3 pathway activation [11]. *This knowledge gap underscores the importance of investigating the effect of sleep on inflammatory biomarkers, including NLRP3, to understand their role in disease development* [12].

This study aims to evaluate the influence of night work on serum NLRP3 inflammasome levels among bakery workers in northwestern Syria, comparing them with a control group of daytime workers. By identifying potential biomarkers and understanding their role in disease pathogenesis, our findings may inform strategies for disease prevention through lifestyle modifications.

II. Material And Methods

Methods:

This cross-sectional study was conducted on workers working in bakeries in northern Syria during the period from May 2021 to September 2023. A total of 78 adults (males) aged 18 years or older participated in this study.

Study Design: Cross-sectional study

Study Location: This study was conducted in northern Syria in the Azaz area, Mare' city - University of Aleppo in the liberated areas - Faculty of Medicine - Department of Clinical Biochemistry.

Study Duration:from June 2021, to September 2023.

Sample size: 78*workers*.

Sample size calculation: The research involving the random selection of 94 healthy male bakery workers in northwestern Syria, aged between 18 and 56 years. Subsequently, 16 workers who met exclusion criteria were excluded, resulting in a study sample of 55-night workers and a control sample of 23 daytime workers, as assessed through a health status evaluation form.

Subjects & selection method:

Night workers in bakeries operate during hours when most people sleep, necessitating constant vigilance without intermittent periods of rest, making them ideal for studying sleep deprivation effects. Informed consent was obtained, and data were collected through personal interviews using the Health Evaluation Questionnaire for Night Workers) [13], ensuring adherence to inclusion criteria are that bakery workers must have six consecutive night shifts per week, and daytime workers six days per week, and the average work duration must be 8 hours.

Inclusion criteria:

- Age between 18-60 years
- Has been working in the bakery for at least three months
- Night shift workers work six consecutive night shifts per week
- Day workers six days per week
- Average working hours must be 8 hours

Exclusion criteria: Any worker who meets the inclusion criteria and does not meet all of the following criteria:

- Non-smoker
- Do not consume alcoholic beverages
- He has not received any vaccine recently
- Do not take any medications at least a week before the blood draw.
- He has no history of cardiovascular disease, kidney or liver problems.
- There is no medical history such as: diabetes, endocrine disorders, psychological disorders, and no previous history of tumors or autoimmune diseases.
- Not a professional athlete.

Procedure methodology

The study group worked 6 days a week for 6-8 hours, starting from 11-12 at night and ending at 7-9 in the morning.

The comparison group worked under similar conditions but during daytime hours, starting from 9-10 in the morning and ending at 6-8 in the evening.

Informed consent was obtained, and data were collected through personal interviews using the Health Assessment Questionnaire for Night Workers, ensuring adherence to inclusion criteria and excluding individuals who met pre-defined criteria

Information collected included demographic details, medical history, smoking status, and work-related characteristics such as shift pattern, duration, and weekly work hours. Day-shift workers were advised to obtain adequate sleep (7-9 hours) before blood collection, while samples from night-shift workers were collected after their shift.

Venous blood samples were collected in gel tubes, allowed to clot for 2 hours, then centrifuged at 2000 rpm for 15 minutes to separate serum, which was stored at -20°C until analysis.Serum NLRP3 levels were measured using semi-automated ELISA kits from Beijing Solarbio Science & Technology Co., Ltd. at the laboratory of the Faculty of Human Medicine, Mare' City, Azaz District, Northern Syria.

Statistical analysis

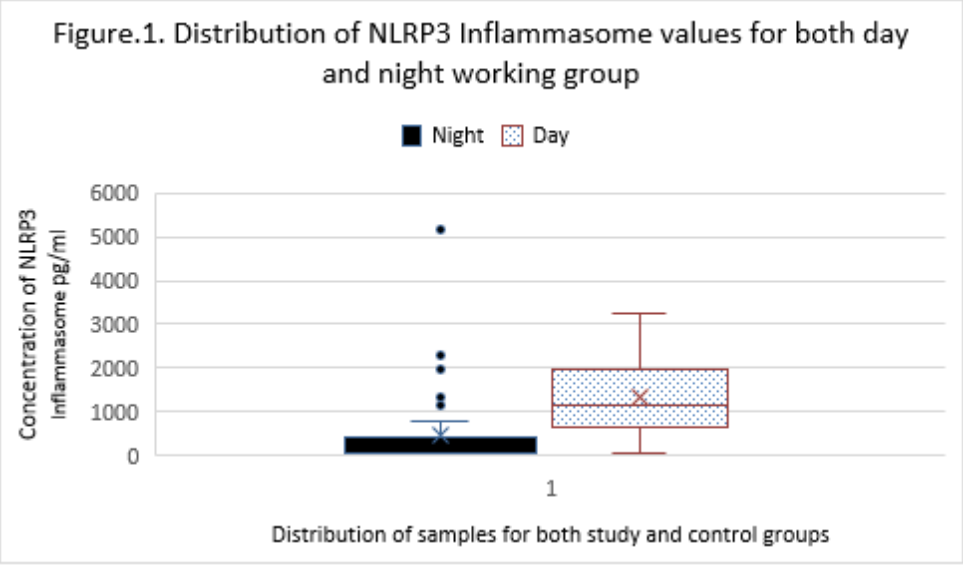
The study sample comprised 78 male bakery workers, with 55 night shift workers and 23 day shift workers. Statistical analysis involved SPSS version 26, ensuring data validity through tests for normal distribution. Non-normally distributed data were transformed, and descriptive statistics were calculated. Independent sample t-tests, one-way ANOVA, and Mann-Whitney U tests were performed, with $p < 0.05$ considered significant. Additionally, analyses of covariance were conducted to adjust for potential confounders affecting NLRP3 levels.

III. Result

The study aimed to evaluate the influence of night work on serum Inflammasome NLRP3 levels compared to day work. Overall comparisons revealed a notable difference in NLRP3 levels between night and day worker groups. Specifically, the average serum Inflammasome NLRP3 level for night shift workers was 454±886 pg/ml (SE = 154), significantly lower than the day shift group's level of 1331±891pg/ml (SE = 85), with a significance level of 0.001 (Table 1). These findings underscore a decrease in NLRP3 levels among night workers relative to their day shift counterparts, indicating statistically significant differences between the two groups.

Table 1- Differences between the study group and the control group

Sample (Total)	*Number*	*Age*	*Years of work*	*Weight*	*Height*	*NLRP3 Inflammasome*
Study Group (Night)	*55*	*29.9±10.6*	*4.3±4.2*	*74.3±9.6*	*171±4.2*	*454±886*
Control Group (Day)	*23*	*32.8±11.1*	*4.13±2.3*	*75.7±8.6*	*168.1±3.1*	*1331±891*



Age Group Analysis:

The study stratified workers into two age groups: those up to 39 years old and those 40 years and above.

It has been found that night shift workers tend to be more at risk of chronic diseases compared to day shift workers, and risk factors vary depending on the age at which night work is performed[14].

In many questionnaire studies, ageing has been found to increase adverse health effects of shiftwork, the critical age being 40-50 years [15]. The limited experimental data on physiological sleep tendency and

performance also suggest that middle aged subjects (over 40 years) would tolerate experimental shiftwork less than younger subjects [16].

Within each age group, comparisons were made between night and day worker groups regarding serum Inflammasome NLRP3 levels. Among workers aged up to 39 years, comprising 41 night workers and 15 day workers, statistically significant differences were found in serum Inflammasome NLRP3 levels (sig=0.001). The mean NLRP3 level for the night shift group was 440±940 pg/ml, while for the day shift group, it was 1452±906 pg/ml (Figure 2). For workers aged 40 years and above, including 14 night workers and 8 day workers. No statistically significant differences were found in serum Inflammasome NLRP3 levels (sig=0.065). The mean serum Inflammasome NLRP3 level for the night shift group was 495±736 pg/ml, compared to 1106±872 pg/ml for the day shift group (Figure 3).

Fig.2: Distribution of NLRP3 Inflammasome values for both the day and night work group under 40 years of age.

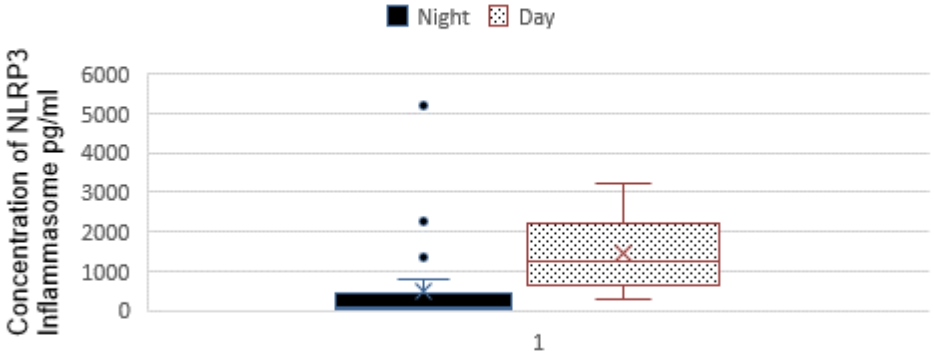
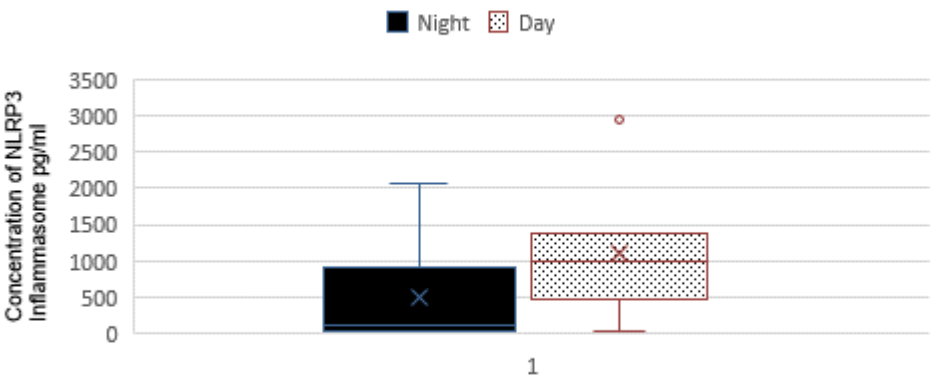


Fig.3: Distribution of NLRP3 Inflammasome values for both the day and night work group more than 40 years of age



Comparison by Years of Work:

Workers were further categorized based on years of work into two groups: up to 3 years, and more than 3 years. Night workers who had three or more years of shift experience showed greater odds of difficulty maintaining sleep[17]. Within each category, comparisons were made between night and day worker groups regarding serum Inflammasome NLRP3 levels. Among workers with 3 years of work experience, comprising 30 night workers and 8 day workers, significant differences were observed in serum Inflammasome NLRP3 levels (sig=0.003). The mean NLRP3 level for the night shift group was 499±1043 pg/ml, compared to 1845±1020 pg/ml for the day shift group (Figure 4). For workers with more than 3 years of work experience, including 25 night workers and 15 day workers, significant differences were found in serum Inflammasome NLRP3 levels

(sig=0.005). The mean NLRP3 level for the night shift group was 399±668 pg/ml, compared to 1057±703 pg/ml for the day shift group (Figure 5).

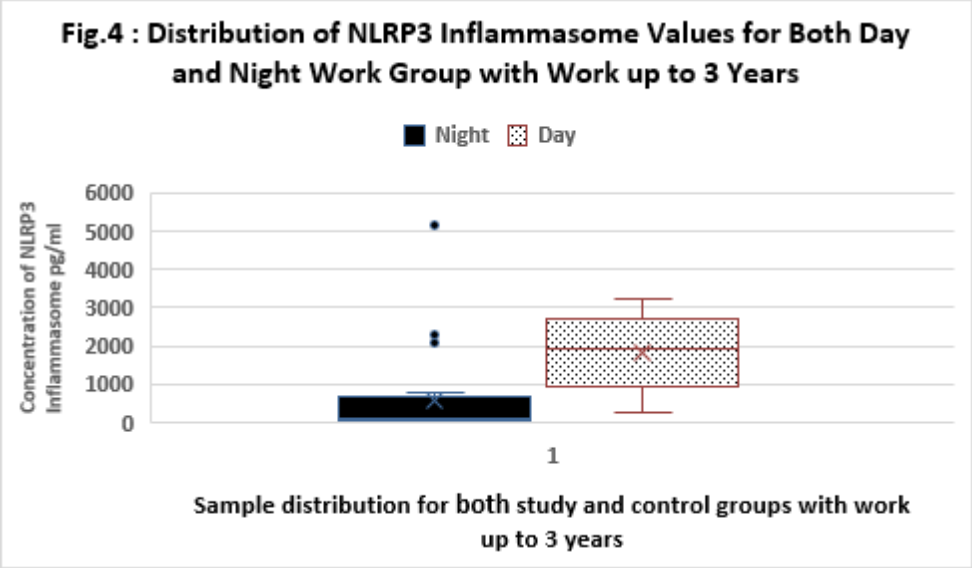


Fig.4 : Distribution of NLRP3 Inflammasome Values for Both Day and Night Work Group with Work up to 3 Years

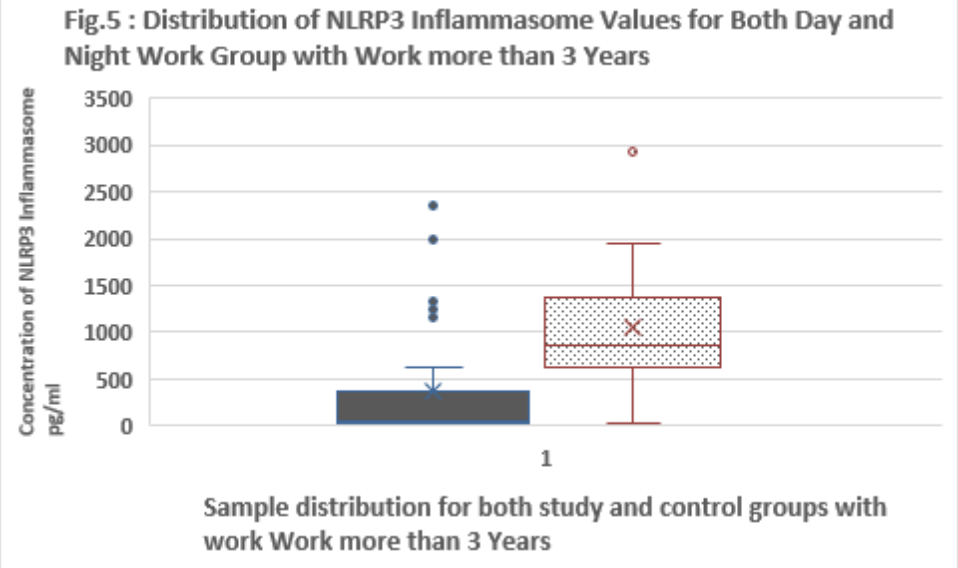


Fig.5 : Distribution of NLRP3 Inflammasome Values for Both Day and Night Work Group with Work more than 3 Years

IV. Discussion

The study investigated the effect of night work on blood levels of Inflammasome NLRP3 compared to day work. The results indicated a significant difference in NLRP3 levels between day and night workers, with night shift workers showing lower levels. Age group analysis revealed clear, statistically significant differences in NLRP3 levels for workers aged less than 40 years, while no statistically significant differences were observed for workers aged 40 years and above. Additional analysis by years of work showed significant differences in NLRP3 levels across different experience categories, with night workers consistently showing lower levels compared to their day-shift counterparts. It is worth noting that among workers with up to 3 years of experience as well as more than 3 years of work experience, significant differences in NLRP3 levels persisted, as statistically significant differences were found in both groups.

According to several studies, exposure to continuous light during the night has been linked to increased oxidative stress, while darkness has been linked to its improvement [18]. Nocturnal action itself has been implicated in the induction of oxidative stress [19], [20], with reactive oxygen species (ROS) acting as a common signal for NLRP3 inflammasome activation [21]. However, the results of our study on decreased NLRP3 levels among night workers are inconsistent with the expected increase in inflammatory values associated with oxidative stress induced by night work [22].

This noticeable decrease in NLRP3 levels among night workers (either in the entire study group, or within the subgroups working up to 3 years and over 3 years, as well as within the age group up to 39 years of

age) can be explained by understanding the body's response to oxidative stress, and its effect on Activation of the NLRP3 inflammasome, and the effect of other factors resulting from night work and sleep deprivation, as various studies indicate an inhibitory effect of factors causing oxidative stress on the NLRP3 inflammasome [23][24], [25]. As is known, cells use antioxidants to combat oxidative stress and protect their biological systems [26]. Notably, Reactive oxygen species (ROS) -induced activation of the NLRP3 inflammasome occurs during priming and is contingent on the presence of a second activating signal [27]. Moreover, ROS and the NLRP3 inflammasome exhibit mutual stimulatory effects, as inhibition of ROS can inhibit NLRP3 expression and activation [28]. [29]. In addition, a crucial point is that the cellular levels of ROS dictate their effects, with low levels acting as signaling molecules that regulate cellular activities, while excess ROS accumulation leads to oxidative stress and cellular damage [30].[31] . This may suggest that oxidative stress in night workers has not reached a sufficient degree to activate the NLRP3 inflammasome, which provides the opportunity for its awareness to increase and decrease, especially if we know that in stressed cells, the formation of stress granules (SGs) acts as a protective mechanism, which may prevent inflammasome activation. NLRP3 [32]،[33]. Competition between stress granules and NLRP3 for essential factors such as DDX3X determines the fate of stressed cells [33] . In addition, regulatory mechanisms such as dopamine signaling, nitric oxide, and autophagy have been identified as inhibitors of NLRP3 inflammasome activation [23],[24] ,[34] ,[35]. Autophagy, in particular, plays a crucial role in suppressing inflammatory responses and reducing tissue injury [35], [36] ،[37]. However, mutations or environmental conditions can induce pathological accumulation of stress granules, which may prevent NLRP3 activation as well [38]. Furthermore, sleep deprivation has been associated with increased oxidative stress, altered dopamine release, and enhanced autophagy activity [39], [40], [41], [42].

As for the lack of differences in the age group of 40 years and above, it may be due to the influence of these people, which may cause a decrease in the efficiency of the adaptive immune function with age [43] and thus a decrease in the effect of the factors that inhibit the activation of the NLRP3 inflammasome mentioned previously, and thus a decrease in it, as it is in the rest of the individuals. From younger ages.

V. Conclusion

Although there are factors that increase the oxidative state of cells in those who work at night or who are deprived of sleep, which are expected to incite the activation of the inflammasome NLRP3, and thus increase its plasma levels, but night work causes lower plasma levels of inflammasome NLRP3 in night workers in our study than those in day workers, and it may help in using it as an indicator for early prediction of the effects of night work on workers' health, except This requires further research and study. Undoubtedly, the process is very complex due to the participation of multiple factors in the immune response, which requires complementary studies to verify the aforementioned conclusions and assumptions.

As a result of the Inflammasome NLRP3 study, we found that there are statistically significant differences between night-shift and day-shift workers, as lower levels of Inflammasome NLRP3 occur in the group of night-shift workers, while there are no statistically significant differences in age-related data between members of the night-shift and day groups. During the day, night shift workers are therefore expected to be more exposed to the risk of decreased Inflammasome NLRP3 as a result of disruption of the inflammatory mechanism within the cells. Therefore, regular follow-up with markers such as Inflammasome NLRP3 in shift workers may be useful and serve as an early indicator in predicting the effects of shift work on the general health of shift workers in general. But this needs more in-depth and broader studies to verify this.

VI. Limitations:

Female workers were not targeted in this research due to the fact that this profession is generally practiced by male workers in Syria. It also targeted one category of night workers, namely bakery workers, in order to ensure that the environmental factors surrounding the working conditions are as similar as possible. Also, the amount of intensity of night lighting to which the workers were exposed at night during their work was not addressed, which may have an effect on the serological marker that was studied.

Declaration Of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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