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Impact of Biological Clock Disturbance on Immune and Physiological Parameters in Kufa Cement Plant Workers

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Abstract
Shift work and environmental exposures are known to be a pivotal factor in immunophysiological dysfunctions, arising from the disruption of circadian rhythms. The objective of this study was to assess the effect of circadian disruption on some inflammatory and hematological markers in workers at the Kufa Cement Plant in Najaf, Iraq, from January to July 2025.
We adopted a cross-sectional analytical approach, categorizing subjects based on their shift schedules (day and night). Blood samples were taken in a uniform manner and used to measure the serum concentrations of IL-6, C-reactive protein CRP, ferritin, and Hb.
This study showed that IL-6, CRP and ferritin concentrations were significantly increased, revealing the presence of systemic inflammatory activation, and hemoglobin concentrations were significantly decreased. Changes were more pronounced in night shift workers compared to day shift workers.
Overall, these results offer robust evidence that circadian misalignment, enhanced by occupational hazards, plays a role in the immune dysfunction, inflammatory iron dyshomeostasis, and the hematological status. These findings highlight a need for greater consideration of circadian health considerations in occupational health programs to reduce long-term health risks in industrial workers

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Introduction

The circadian system is an endogenous time-keeping mechanism that governs various physiological and immune functions over a ~24-hour period, predominantly driven by the master clock in the suprachiasmatic nucleus of the hypothalamus. It coordinates peripheral clocks in peripheral tissues via neural and hormonal pathways to drive optimal coordination of metabolic, endocrine and immune events [1, 2]. Circadian rhythm disruption, as seen in shift workers, has emerged as a key driver of systemic dysfunction and susceptibility to diseases [3].
Circadian misalignment coupled with workplace hazards, such as particulate matter, chemical exposure and physical strain, makes industrial workers, especially those in high-exposure industries such as cement factories, particularly vulnerable [4, 5]. Shift work, particularly night shifts and rotating shifts, causes a misalignment between the intrinsic circadian time structure and environmental time cues, leading to changes in sleep-wake cycles, hormone levels, and immune function [6].
Recent research has also highlighted the strong association between circadian misalignment and chronic low-grade inflammation, marked by sustained elevations in inflammatory markers such as IL-6 and CRP [7]. IL-6 is a multifunctional cytokine that plays a role in immune response, acute-phase reaction, and inflammation, and CRP is a classic acute-phase protein produced by the liver in response to inflammation [8].

Elevated levels of these markers have been linked to the development of cardiovascular diseases, metabolic syndrome, and compromised immune responses, especially in subjects exposed to shift work [9].

Besides inflammatory markers, shifts in circadian rhythm have been observed to affect hematological indices. Hb, which represents the oxygen-carrying capacity of the blood, might be reduced by oxidative stress, inflammation and dysregulated erythropoiesis secondary to irregular sleep-wake cycles [10]. On the other hand, intra-cellular iron storage protein and acute-phase reactant, ferritin, is frequently elevated in inflammatory states due to changes in iron metabolism and immune response [11]. Interactions between inflammation and iron metabolism are now recognised as important aspects of chronic disease.

While there is increasing global interest in the field of circadian biology, there is a lack of information relating to its effects on immune and physiological factors among workers in the developing world, especially in the Middle East. Cement plant workers in Iraq are exposed to not only shift work but also environmental pollutants that can further impact inflammatory and hematological changes. Thus, it is important to explore the interactive effects of circadian misalignment and occupational exposures to better understand potential health risks in this group [12].

This research seeks to assess the influence of circadian rhythm misalignment on a range of immune and physiological parameters - IL-6, CRP, hemoglobin and ferritin - in workers at the Kufa Cement Plant in Najaf, Iraq. Through comparisons of these variables across various work cycles (day, night, and rotating shifts), this study aims to shed light on the mechanisms that link circadian disruption to systemic inflammation and hematological alterations, thus guiding occupational health interventions and preventive measures.

Materials and Methods

Study Design and Setting

An analytical cross-sectional study was carried out from January to July 2025 at the Kufa Cement Plant, Najaf Governorate, Iraq. The aim was to assess the effects of circadian rhythm desynchronisation on some inflammatory and hematological markers in industrial workers subjected to various working shifts.

Study Population and Grouping

In this study, 84 workers from the cement plant were recruited. The workers were divided into two shift groups: day shift (n = 42) and night shift (n = 42). This stratification was used to assess the biological effect of circadian misalignment due to night shift work.

Eligibility Criteria

To be included, participants had to be adult workers (20-60 years), with at least one year of employment, who worked on a fixed shift schedule (either day or night shift). Written informed consent was obtained from all participants.

Participants were also excluded if they had a history of chronic inflammatory or autoimmune disorders, recent acute infections (within the past 14 days), hematological disorders or anemia unrelated to their occupational exposure, or chronic systemic diseases (diabetes mellitus, cardiovascular diseases, or hepatic diseases). Participants were also excluded if they were taking anti-inflammatory or immunosuppressive medications or had received blood transfusions in the last three months.

Ethical Considerations

The research was carried out in line with the principles of the Declaration of Helsinki. The study was approved by the Institutional Review Board (IRB) of the College of Science, University of Kufa, as well as the administration of Al-Sadr Medical City and Kufa Cement Plant before the start of the study.

Participants were provided with comprehensive information on the study aims, methods and risks. Participants provided written consent before participating. Privacy was maintained by anonymising data and limiting access to the research team.

Sample Size Consideration

The sample size (n = 84) was based on availability and feasibility during the time frame of the study. Although no formal sample size calculation was performed, the sample size was considered adequate for detecting significant differences between groups based on prior similar studies, the sample was considered adequate for detecting significant differences between groups based on prior studies. for comparison between groups.

Data and Sample Collection

Demographic and work history information (age, years of employment, work shift) was collected through a questionnaire.

We collected venous blood samples (5 mL) under controlled conditions. To control for diurnal variations, samples were collected in relation to the worker's shift (i.e., day shift was considered as light phase). Blood was left to clot at room temperature, centrifuged to obtain the serum fraction, and then frozen at −20 °C.

Laboratory Analysis

Serum IL-6 levels were determined using commercially available enzyme-linked immunosorbent assay ELISA kits (e.g., MyBioSource, USA), following the manufacturer's protocol. CRP concentrations were also measured by ELISA.

Serum ferritin concentrations were determined using an automated immunoassay analyzer (Cobas c111, Roche Diagnostics, Germany) according to the manufacturer's instructions. Hb levels were measured in an automated hematology analyzer (Sysmex XN-series, Japan) following established protocols.

All measurements were made in duplicate. Low and high-concentration quality control samples were run in each assay and all measurements were carried out according to routine laboratory protocols for ensuring accuracy, repeatability, and reproducibility of the data.

Statistical Analysis

Data were analysed with an appropriate statistical software package (SPSS version 25 or similar). Continuous data were tested for normal distribution with the Shapiro-Wilk test.

Normally distributed continuous variables were presented as

mean ± standard deviation (SD) and tested for differences between groups using the independent samples t-test. However, non-normally distributed variables were reported as median (interquartile range, IQR) and the Mann-Whitney U test was used to compare groups**.**

Two-tailed tests were used and p-value below 0.05 was regarded as statistically significant.

Results

1. Hemoglobin Hb Levels in Relation to Shift Work

An independent samples t-test was applied to compare hemoglobin levels between day and night shift workers. Results are expressed as mean ± SD. We found a significantly lower Hb levels in night shift workers compared to day shift workers ($P < 0.0001$).

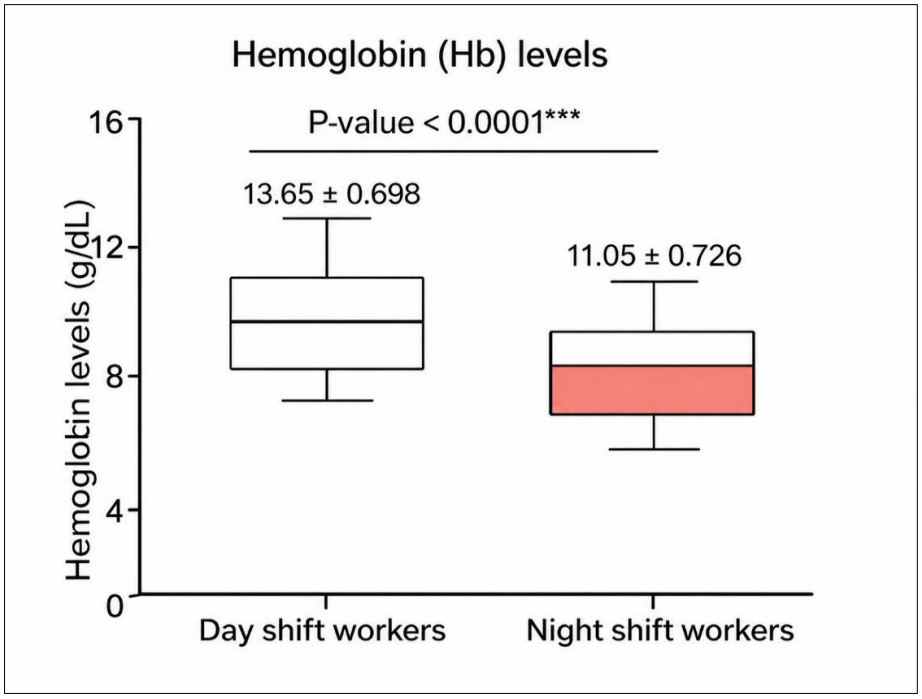

Fig 1: Comparison of serum hemoglobin levels between day and night shift workers. Data are presented as mean ± SD.

2. Ferritin Levels in Relation to Shift Work

The serum ferritin levels of day shift workers (control) and night shift workers (patient) were compared. The results are expressed as mean ± SD. There was a significant increase in ferritin levels in night shift workers compared to day shift workers ($P < 0.0001$).

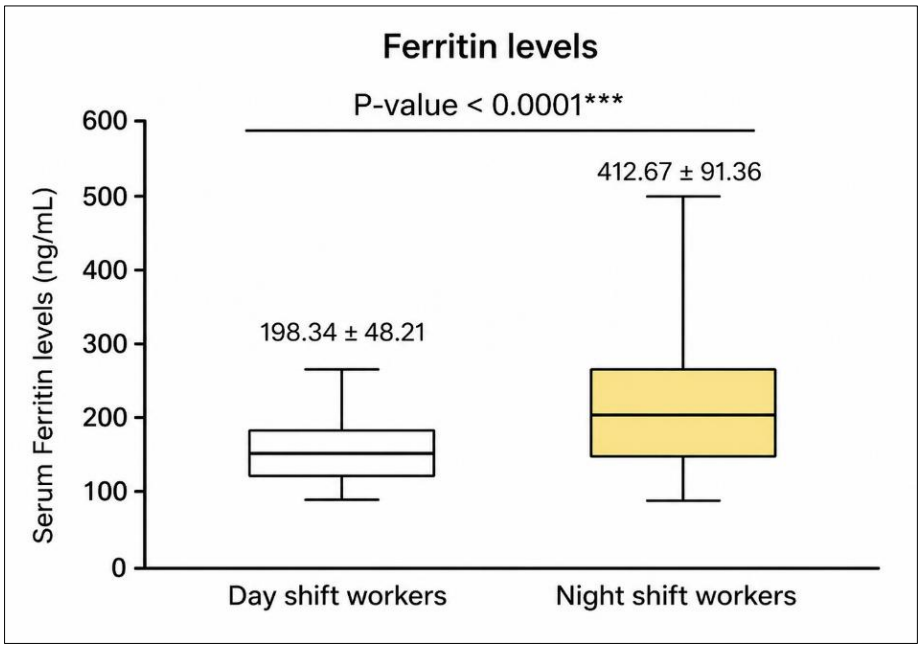

Fig 2: Comparison of serum ferritin levels between day shift and night shift workers.

3. C-reactive Protein CRP Levels in Relation to Shift Work

Day shift workers (control) and night shift workers (patients) were compared for their serum levels of CRP. Results are expressed as mean ± SD. There was a significant increase in CRP concentrations in night shift workers compared to day shift workers (P < 0.0001).

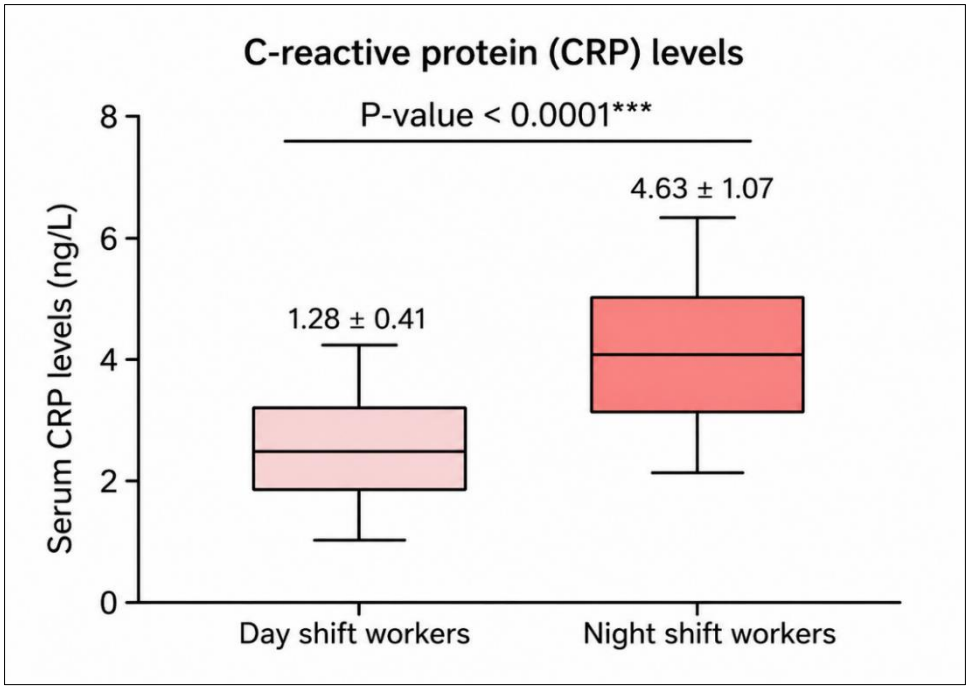

Fig 3: Comparison of serum C-reactive protein (CRP) levels between day shift and night shift workers

4. IL-6 Levels in Relation to Shift Work

IL-6 levels in the blood of day shift workers (control) and night shift workers (patients) were compared. Results are expressed as mean ± standard deviation (SD). IL-6 levels in night shift workers were significantly higher than in day shift workers (P < 0.0001).

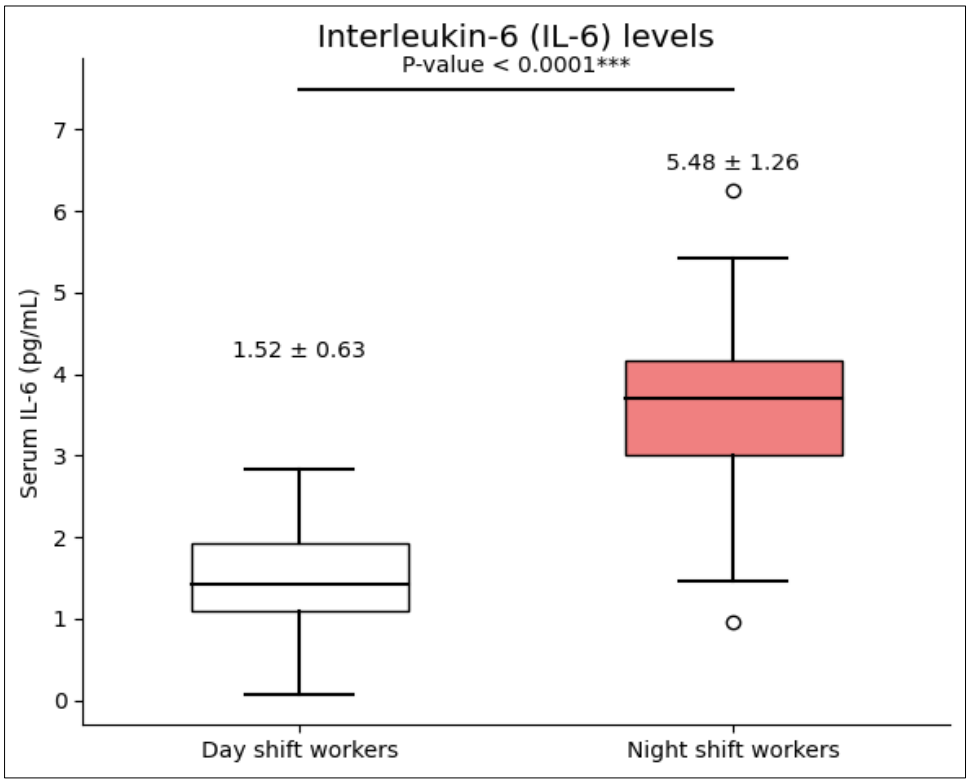

Fig 4: Comparison of serum interleukin-6 (IL-6) levels between day shift and night shift workers.

Discussion

The present findings provide compelling evidence that circadian rhythm disruption in cement plant workers is significantly rhythm disruption in cement plant workers is significantly associated with systemic inflammatory activation and hematological alterations.

in cement plant workers to be strongly linked to systemic inflammatory activation and blood changes. Our results revealed significant increases in IL-6, CRP and ferritin concentrations, and a decrease in Hb levels among night shift workers. These findings collectively confirm the hypothesis that circadian rhythm disruption, coupled with occupational hazards, is involved in immune dysregulation, sub-clinical inflammation and homeostatic dysregulation.

One of the notable findings of this study is the elevated levels of IL-6 in night shift workers. IL-6 is a key pro-inflammatory cytokine that regulates immune responses and the acute-phase reaction, and its increase indicates sustained immune activation. Circadian disruption has been shown to alter circadian rhythmicity of cytokines and to compromise immune functions [13, 14]. Experimental and clinical evidence has shown that sleep deprivation and circadian misalignment result in increased IL-6 production via activation of the nuclear factor kappa B (NF-κB) pathway, contributing to chronic inflammation [15]. This is consistent with our current results, which show a significant increase in IL-6 levels in night shift workers ($p < 0.0001$), reflecting circadian misalignment-related inflammation.

Likewise, night shift workers had elevated CRP levels, also indicating activation of systemic inflammation. CRP is produced in the liver in response to IL-6 and is considered a valuable marker of subclinical inflammation [16]. The simultaneous increase in IL-6 and CRP observed in our study indicates activation of the IL-6-CRP inflammatory axis, which has been shown to play a role in the development of cardiovascular and metabolic diseases [17]. Environmental factors, such as exposure to particulate matter and pollutants in the workplace, may further contribute to the inflammatory process, especially in cement factories [18].

Another key finding is the higher ferritin levels in night shift workers. While its primary role is as an intracellular iron storage protein, ferritin is also an acute-phase protein that rises during inflammation [19]. The raised ferritin levels in our study are likely due to iron sequestration during inflammation rather than iron overload. This is achieved by cytokine-mediated induction of hepcidin, particularly via IL-6, leading to iron sequestration and ferritin elevation, which is a characteristic feature of the iron dysmetabolism seen in inflammation [20]. Our results are in line with previous studies reporting circadian disruption-associated iron changes [21].

Conversely, hemoglobin levels were lower in night shift workers. This could be due to suppression of erythropoiesis via inflammatory pathways (anemia of inflammation). Cytokines like IL-6 and TNF-α can suppress erythropoietin levels and bone marrow activity, resulting in a reduction in red blood cell production [20, 22]. Furthermore, the loss of circadian rhythm can disrupt the regulation of the hematological process and lead to oxidative stress, which may also contribute to lower hemoglobin [23]. The negative association between inflammatory biomarkers and hemoglobin levels is consistent with the idea of inflammation-induced hematopoietic dysfunction.

Overall, the simultaneous increase in IL-6, CRP and ferritin, together with a decrease in hemoglobin levels, suggests a plausible biological link between circadian disruption, immune activation, iron metabolism and hematological impairment. These results suggest that shift work is a major biological challenge that, in addition to sleep disruption, impacts several physiological processes.

These changes may influence the risk of chronic disease such as cardiovascular disease, metabolic syndrome and fatigue in the clinical and occupational health setting. As such, these results highlight the need for proactive measures, including shift scheduling, biomarker surveillance, and strategies to mitigate circadian misalignment and occupational hazards.

Conclusion

In summary, the current study offers robust evidence of an association of circadian dysregulation in cement plant workers with systemic inflammatory response and alterations in hematological indices. The night shift was related to elevated concentrations of IL-6, CRP, and ferritin and a decrease in hemoglobin.

This evidence supports the hypothesis that circadian misalignment is an important factor in the development of immune dysfunction, inflammation-induced iron dyshomeostasis and physiological dysfunction. For occupational health, the findings underline the need to consider circadian health in strategies such as shift scheduling and routine biomarker assessments to reduce the long-term health burden and enhance work productivity.

This study provides novel insights into the combined impact of circadian disruption and occupational exposure on inflammatory and hematological biomarkers in an underrepresented population. The use of multiple biomarkers and controlled sampling conditions enhances the robustness and reliability of the findings.

Limitations

Although the current study has many merits, there are some limitations. First, the cross-sectional nature of the study prevents the determination of causal links between circadian disruption and changes in biomarkers. Second, the sample size ($n = 84$) was relatively small, which may limit the applicability of the results to larger populations.

Third, the single time point for biomarker measurements may not reflect circadian variation in inflammatory and hematological markers. Moreover, several potential confounders, such as sleep duration, body mass index, smoking, diet, and exposure levels, were not fully adjusted for.

Additional longitudinal research with larger cohorts and sophisticated multivariable analyses is needed to more fully understand temporal associations, as well as the mechanisms that underpin the potential health effects of circadian disruption.

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