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Study Protocol

Integrated Assessment of Night Shift Work and Chemical Exposure in Female Healthcare Workers: A Study Protocol

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Abstract: Night shift work (NSW) is common among healthcare workers (HCW) and can disrupt circadian rhythms, leading to metabolic dysregulation, systemic inflammation, and increased breast cancer risk in women. In hospital settings, NSW often co-occurs with exposure to disinfectants and sterilants, creating combined circadian and chemical stress. Female HCW may be particularly vulnerable due to sex-specific endocrine factors and cumulative chemical exposures. This prospective study protocol proposes an integrated occupational health surveillance model comparing night and day shift female HCW. The protocol combines clinical evaluation, molecular biomarker profiling, lifestyle and psychosocial assessment, as well as environmental monitoring of key chemical exposures. Participants will undergo baseline assessment, a 1-month follow-up to monitor adherence to personalized health recommendations, and a 12-month follow-up to evaluate changes in clinical, metabolic, molecular, and lifestyle parameters. The multidimensional approach aims to clarify the interactions between circadian disruption and chemical exposures, identify early biomarkers of risk, and help the development of targeted interventions to improve metabolic health, reduce chemical burden, and support circadian alignment and overall well-being in female HCW.

Keywords: night shift work; healthcare workers; circadian disruption; chemical exposures; occupational health

1. Background and Rationale

Night shift work (NSW) is common in healthcare sector and it can induce chronic circadian misalignment, disrupting neuroendocrine regulation, metabolism, immune function, and DNA repair, and increasing the risk of sleep disturbances, metabolic syndrome, inflammation, and potentially breast cancer (BC) in women [1,2]. Healthcare Workers (HCW) are particularly vulnerable due to long-term shift schedules, high workload, psychosocial stress, and irregular patterns of sleep, diet, and physical activity patterns [3].

NSW interferes with the central circadian clock in the hypothalamus, affecting hormone secretion, metabolism, and cellular homeostasis [2]. In women, nocturnal light exposure can reduce melatonin, impair estrogen regulation, antioxidant defenses, and DNA repair, providing a mechanistic link to BC risk. The International Agency for Research on Cancer (IARC) classifies NSW as "probably carcinogenic to humans" (Group 2A) [4].

Novel molecular players, including clock genes (BMAL1, PER2, CRY1) and circadian microRNAs (miRNAs), as well as extracellular vesicles (EVs), may serve as early biomarkers of circadian-related biological stress [5,6].

In parallel, HCW are exposed to chemicals including disinfectants (quaternary ammonium compounds, glutaraldehyde), sterilants (volatile organic compounds), cytotoxic drugs, and airborne pollutants [7]. These

exposures contribute to oxidative stress, DNA damage, inflammation, and long-term health risks, with women disproportionately affected due to occupational roles, physiology, and susceptibility to endocrine disruptors [8–10].

NSW and chemical exposures are key components of the healthcare occupational exposome, yet their combined biological effects remain poorly characterized [11]. Integrated strategies combining biological monitoring, environmental assessment, and molecular analyses allow evaluation of these multifactorial risks and identification of early biomarkers predictive of health impairment [12,13].

This study protocol proposes a structured occupational health surveillance program in female HCW, integrating baseline molecular, clinical, lifestyle, and psychosocial assessments. Follow-up at one month (T1) will evaluate compliance with personalized recommendations, and a 12-month follow-up (T12) will assess long-term changes in molecular, clinical, and behavioral parameters. The goal is to reduce health risks, improve circadian alignment, mitigate chemical exposures, and promote overall well-being in female HCW.

2. Objectives

2.1. Primary Objectives

- To determine the combined effects of NSW and occupational chemical exposures on metabolic, inflammatory, hormonal, circadian, and BC-related biomarkers in female healthcare workers.
- To quantify internal chemical exposure and examine associations with early biological effect markers.
- To characterize environmental contamination and identify occupational determinants of exposure.
- To assess circadian disruption and its relationship with shift patterns, chemical exposures, metabolic changes, and BC-related molecular signatures.

2.2. Secondary Objectives

- To describe comprehensive inflammatory, metabolic, hormonal, lipid, and oxidative stress profiles associated with circadian misalignment and chemical exposures.
- To evaluate anthropometric, body composition, cardiovascular, and organ-specific markers in relation to systemic inflammation and circadian disruption.
- To assess lifestyle and psychosocial factors (diet, physical activity, sleep, chronotype, psychological well-being, work stress, work ability) and integrate these with biological and environmental data.

2.3. Intervention Objectives

- To provide personalized health advice targeting nutrition, sleep hygiene, physical activity, circadian alignment, and chemical risk reduction.
- To monitor adherence through structured follow-up and behavioral diaries.
- To assess changes in clinical, biochemical, lifestyle, circadian, and exposure parameters at 12 months relative to baseline.

2.4. Exploratory Objectives

- To investigate EVs as integrative biomarkers of systemic stress, circadian disruption, metabolic impairment, and early BC-related alterations.
- To develop multi-layer predictive models combining environmental, biological, circadian, metabolic, and behavioral data to identify high-risk HCW.
- To evaluate the feasibility and added value of incorporating chemical risk assessment into occupational health surveillance for female night shift workers.

3. Study Population and Criteria

The study population will consist of female HCW employed at a university hospital in southern Italy. Eligible participants will be aged 25–65 years and have at least six months of continuous employment at the hospital. Participants will be stratified into two groups based on shift exposure: NSW, performing ≥3 night shifts per week for ≥1 year, and day-shift workers (DSW), with no night shift exposure for at least one year.

This recruitment framework captures two overlapping occupational risk domains: (1) organizational factors, including shift patterns, rotation type, workload, and psychosocial stress, contributing to circadian disruption and behavioral changes; and (2) chemical exposures, based on tasks and areas involving disinfectants or sterilants. To ensure representation of differing chemical exposure profiles, recruitment will target multiple hospital

departments, such as wards, operating theatres, sterile processing units, laboratories, oncology units, and cleaning services (Supplementary Table S1).

3.1. Inclusion Criteria

- Female sex, age 25–65 years.
- Employed at the study hospital for ≥6 months.
- For NSW group: ≥3 night shifts/week for ≥1 year.
- For DSW group: no night shift exposure for ≥1 year.
- Willing and able to provide informed consent and comply with study procedures.

3.2. Exclusion Criteria

- History of active cancer within the previous five years.
- Current pregnancy or breastfeeding.
- Chronic use of medications substantially affecting circadian/hormonal status (e.g., chronic systemic corticosteroids, regular melatonin supplements, non-standard hormonal therapies).
- Severe psychiatric disorder or cognitive impairment precluding valid participation.
- Acute infection or inflammatory condition at baseline.
- Any condition judged by the investigator to preclude safe participation.

4. Stratification, Covariates and Occupational Risk

Participants will be stratified by shift status (NSW vs. DSW). Occupational covariates will capture organizational risk, including total years of shift work, rotation type and frequency, weekly night shifts, role/department, involvement in disinfection or sterile processing, use of PPE, and psychosocial factors (e.g., effort–reward imbalance, work ability index).

Chemical risk will be assessed through occupation-specific exposure histories, biological monitoring (urinary and blood biomarkers), and environmental sampling (personal and area air, surface wipes). Department and task assignments will define exposure clusters for analysis, allowing disentangling of organizational versus chemical effects.

5. Sample Size

The primary objective is to detect differences in biomarkers of circadian disruption and chemical-related effects between NSW and DSW. Based on a moderate effect size, 64 participants per group provide 80% power at $\alpha = 0.05$; accounting for attrition, the target is 75 per group (total $N = 150$). Secondary and subgroup analyses may be underpowered and will be considered exploratory, with confidence intervals and effect estimates reported. A blinded variance check may be performed if observed variability differs from initial assumptions.

6. Recruitment, Representativeness and Retention

Recruitment will target multiple hospital departments to capture variability in organizational and chemical exposures, using briefings, mailing lists, and targeted invitations. Follow-up will be flexible and aligned with shift schedules to minimize attrition. Reasons for non-participation and loss to follow-up will be documented. Missing data will be addressed using appropriate statistical methods (e.g., multiple imputation), with sensitivity analyses reported.

7. Study Design

This is a prospective cohort study integrating multidisciplinary assessments to evaluate the combined impact of NSW and chemical exposures in female HCW. Participants will be stratified into NSW and DSW and undergo a comprehensive baseline assessment (T0) including clinical, anthropometric, biochemical, molecular, occupational, and lifestyle evaluations. Biological samples (blood, plasma, serum, urine) will be collected to analyze circadian (clock genes, circadian miRNAs), inflammatory, oxidative stress, DNA damage, metabolic, hormonal, and chemical exposure biomarkers (urinary metabolites of QACs, glutaraldehyde, VOCs, hemoglobin adducts).

Occupational exposure will be characterized via work history, shift patterns, departmental assignment, and environmental monitoring (surface and air sampling). Lifestyle and psychosocial factors will be assessed through validated questionnaires and structured daily diaries to capture real-time behaviors over representative work cycles.

Participants will receive individualized health advice targeting circadian alignment, lifestyle optimization, and chemical exposure mitigation. Early follow-up at one month (T1) will assess adherence, and a 12-month follow-up (T12) will reassess all parameters to evaluate longitudinal changes and early biological effects. This

integrated design allows the multidimensional assessment of exposures and outcomes within an occupational exposome framework (Figure 1).

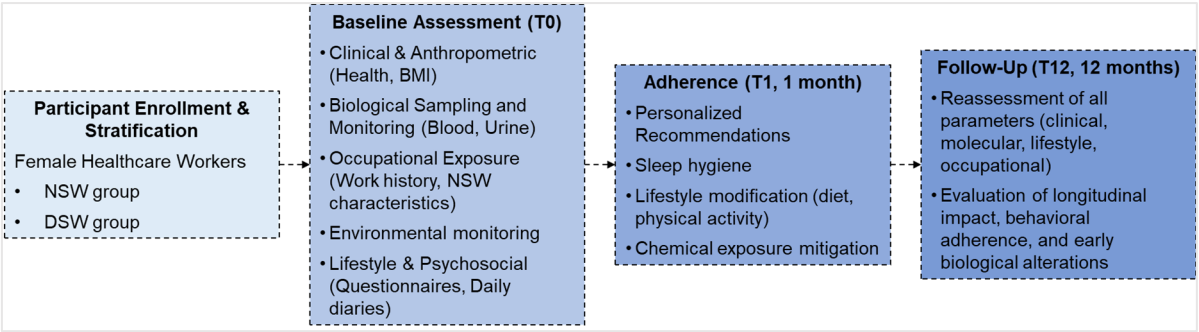

Figure 1. Study design overview. Female healthcare workers are stratified by work schedule (night-shift, NSW vs. day-shift, DSW) and undergo a baseline assessment (T0) including clinical evaluation, anthropometrics, lifestyle, occupational exposure, and biomarker sampling. Participants receive personalized health advice (T1, 1 month), and all assessments are repeated at 12 months (T12) to evaluate longitudinal changes, adherence, and early biological effects.

8. Informed Consent

All participants will receive verbal and written information regarding study objectives, procedures, risks, and benefits, and provide written informed consent prior to enrollment. Participation is voluntary, with the right to withdraw at any time without consequences. Confidentiality will be strictly maintained, with coded data stored securely.

9. Ethical Approval

The study has been approved by the Ethics Committee of the university hospital and complies with national and international guidelines, including Good Clinical Practice. Any protocol modifications will require formal approval. Biological sample collection, storage, and analysis will adhere to local regulations.

10. Statistical Analyses

Descriptive statistics will summarize demographic, occupational, clinical, molecular, lifestyle, and chemical exposure data. Continuous variables will be expressed as mean ± SD or median (IQR), categorical variables as counts and percentages. Between-group comparisons (NSW vs. DSW) will use *t*-tests or Mann–Whitney U tests for continuous variables and chi-square or Fisher's exact tests for categorical variables.

Multivariate analyses (linear and logistic regression) will assess associations between shift work, chemical exposures, molecular biomarkers, lifestyle factors, and health outcomes, adjusting for confounders (age, BMI, menopausal status, smoking). Mixed-effects models will evaluate intra-individual changes across T0, T1, and T12. Chemical biomarker data will be log-transformed as needed, with correlation analyses to examine relationships between exposure levels, molecular signatures, lifestyle behaviors, and circadian disruption. Exposure-response modeling may be performed to identify dose-dependent effects. Statistical significance will be set at $\alpha = 0.05$. Analyses will be conducted using validated software (e.g., SPSS, GraphPad Prism).

11. Health Surveillance

11.1. Health Assessment

At baseline (T0), participants undergo a multidisciplinary health evaluation including anthropometrics, body composition, cardiovascular parameters (blood pressure, ECG), and general health indicators. Fasting blood samples (≥8 h) are collected for glucose, cholesterol, and other biochemical markers. Smoking status is categorized as current, former (<12 months), or never (Supplementary Table S2).

11.2. Medical History

Female participants provide personal and family medical history, lifestyle factors (smoking, alcohol), and reproductive history (pregnancy, menopause, hormonal therapy, parity, abortions). Previous cancer diagnoses and current medications are documented (Supplementary Table S3).

11.3. Occupational History

Structured questionnaires capture both organizational (shift work) and chemical exposure dimensions, including: duration of employment, shift schedules, department-specific tasks, frequency and type of disinfectant use (QACs, glutaraldehyde, VOCs), cleaning workloads, ventilation, PPE use, perceived exposure intensity, and chemical-related symptoms (Supplementary Table S4).

12. Blood and Urine Tests

At baseline (T0), participants undergo fasting blood and urine collection in the morning. Routine clinical chemistry is performed to assess metabolic, hepatic, renal, and hormonal parameters, while urine samples are analyzed for circadian, oxidative stress, and chemical exposure biomarkers.

All samples are collected after an overnight fast of ≥8 h. Night shift workers are sampled following a standardized recovery interval to ensure comparability. Blood is drawn by trained personnel using contaminant-free materials. PBMCs are isolated for circadian gene expression and DNA damage analysis, while plasma and serum are used to quantify circadian miRNAs, inflammatory markers, oxidative stress indicators, hormones (melatonin, cortisol, thyroid hormones, vitamin D), metabolic markers, and extracellular vesicles. Urine is collected as the first morning void for measurement of melatonin and cortisol metabolites, oxidative DNA damage markers (e.g., 8-OHdG), and metabolites of QACs, glutaraldehyde, and VOCs. All samples are stored at −80 °C in a dedicated biobank under GLP conditions. Supplementary Tables S5 and S6 summarize the biomarkers analyzed in participants' specimens.

13. Indexes Calculation

As reported in Table 1, a comprehensive set of clinical, metabolic, and body composition indexes will be derived from anthropometric measurements, laboratory parameters, and molecular biomarkers to evaluate cardiometabolic health, physiological stress, and early biological effects associated with NSW and chemical exposures (QACs, glutaraldehyde, and VOCs).

Table 1. Derived clinical and metabolic indexes.

Category	Index/Ratio	Definition/Formula	Reference
Cardiovascular Risk	IRCV	IRCV = 100 × (1 − 0.889^(–RF)); RF = [ln(E) × 3.06] + [ln(C-T) × 1.12] − [ln(C-HDL) × 0.93] + [ln(PAS) × AIT] + S + D − 23.98; E = age; C-T = total cholesterol; C-HDL = HDL cholesterol; PAS = systolic blood pressure; AIT = antihypertensive therapy (0/1); S = sex (0 female/1 male); D = diabetes (0/1)	[14]
Lipid-Related Ratios	LDL/HDL ratio	LDL cholesterol/HDL cholesterol	-
	Total Cholesterol/HDL ratio	Cardiometabolic risk indicator	-
	Triglycerides/HDL ratio	Surrogate marker of insulin resistance and atherogenic dyslipidemia	-
Metabolic Indexes	HOMA-IR	(Glucose × Insulin)/405 (glucose in mg/dL)	[15]
Liver Health	AST/ALT ratio	Marker of hepatic injury; inversion (<1) suggests NAFLD	-
	FIB-4 Index	(Age × AST)/(Platelets × √ALT)	[16]
Oxidative Stress & DNA Damage	Urinary 8-OHdG Index	8-OHdG/urinary creatinine (adjusted for dilution)	[17]
	Total Antioxidant Status (TAS)	Overall plasma antioxidant capacity (mmol/L)	[18]
Body Composition Indexes	FFMI	Fat-Free Mass/height^2 (m^2)	-
	BCMI	Body Cell Mass/height^2 (m^2)	-
	SMI	Appendicular Skeletal Muscle Mass/height^2 (m^2)	-

Cardiovascular risk will be estimated using the Italian Progetto Cuore individual risk score (IRCV), developed by the Istituto Superiore di Sanità. The IRCV calculates the 10-year probability of a first major cardiovascular event (myocardial infarction or stroke) using eight continuous risk factors: age, sex, diabetes status, smoking, systolic blood pressure, total cholesterol, HDL cholesterol, and antihypertensive therapy. Its continuous-variable design and incorporation of HDL cholesterol and treatment status provide a precise, individualized estimate for adults aged 25–69 years without prior cardiovascular disease. The score is not applicable to pregnant women or individuals with extreme blood pressure (>200 mmHg or <90 mmHg) or cholesterol levels (total cholesterol >320 mg/dL or <130 mg/dL; HDL <20 mg/dL or >100 mg/dL). Laboratory parameters must be obtained using standardized methods within three months of assessment. Follow-up frequency is stratified by

baseline risk: every six months for high-risk individuals (≥20%), annually for moderate risk (3–19.9%), and every five years for low risk (<3%), with interpretation by a qualified physician.

Additional metabolic and liver indexes include HOMA-IR, AST/ALT ratio, and FIB-4 score. Lipid-related ratios (LDL/HDL, total cholesterol/HDL, triglycerides/HDL) will be calculated from fasting lipid profiles. Body composition will be evaluated using BMI, FFMI, BCMI, and SMI.

Given the relevance of oxidative stress and DNA damage as early indicators of biological responses to combined circadian disruption and chemical exposures, derived indices such as urinary 8-OHdG (creatinine-adjusted) and systemic Total Antioxidant Status (TAS) will support mechanistic interpretation of cardiometabolic and occupational risk profiles.

14. Questionnaire-Based Assessments

As reported in Table 2, participants will complete a structured battery of validated questionnaires to assess lifestyle behaviors, circadian health, psychological well-being, work-related functioning, and occupational exposures relevant to both NSW and chemical hazards. Physical activity will be evaluated using the International Physical Activity Questionnaire (IPAQ), and dietary habits and adherence to the Mediterranean diet will be assessed through the Medi-Lite score. Sleep quality, daytime sleepiness, and chronotype will be measured with the Pittsburgh Sleep Quality Index (PSQI), the Epworth Sleepiness Scale (ESS), and the Morningness–Eveningness Questionnaire (MEQ), providing an integrated evaluation of sleep–wake patterns and circadian alignment in relation to shift work.

Table 2. Questionnaire-based assessments: scoring systems and interpretation.

Domain	Instrument	Scores/Categories	Interpretation	Reference
Physical Activity	IPAQ—International Physical Activity Questionnaire	Low (<600 MET-min/week), Moderate (600–3000 MET-min/week), High (>3000 MET-min/week)	Classifies total weekly physical activity across work, transport, domestic, and leisure domains; higher category = greater activity and better adherence	[19]
Dietary Adherence	Medi-Lite Score	0–18 points: 0–7 = Low, 8–11 = Moderate, 12–18 = High	Higher scores indicate stronger adherence to the Mediterranean diet pattern	[20]
Sleep Quality	PSQI—Pittsburgh Sleep Quality Index	0–21: 0–5 = Good, >5 = Poor	Reflects sleep duration, latency, disturbances, and efficiency; higher scores = worse sleep quality	[21]
Daytime Sleepiness	ESS—Epworth Sleepiness Scale	0–24: 0–10 = Normal, 11–12 = Mild EDS, 13–15 = Moderate EDS, 16–24 = Severe EDS	Measures likelihood of dozing in everyday situations; higher scores = more excessive daytime sleepiness	[22]
Chronotype	MEQ—Morningness-Eveningness Questionnaire	16–86: 70–86 = Definitely morning, 59–69 = Moderately morning, 42–58 = Intermediate, 31–41 = Moderately evening, 16–30 = Definitely evening	Assesses circadian preference, determining chronotype and sleep-wake orientation	[23]
Psychological Well-being	WHO-5 Well-Being Index	0–25: <13 = Possible depression/low well-being	Higher scores = better well-being; score <13 suggests need for further evaluation	[24]
Work-Related Stress	ERI—Effort-Reward Imbalance	ERI Ratio = Effort/Reward: >1 = High stress, =1 = Neutral, <1 = Favorable balance	Assesses mismatch between effort invested and rewards received; includes overcommitment subscale	[25]
Work Ability	WAI—Work Ability Index	7–49: 7–27 = Poor, 28–36 = Moderate, 37–43 = Good, 44–49 = Excellent	Evaluates perceived work ability relative to job demands, physical and mental health, and functional capacity	[26]
Chemical Exposure	CEQ—Chemical Exposure Questionnaire	Frequency and intensity scoring per chemical, task, and duration	Captures occupational exposure to disinfectants, sterilants, and VOCs, supporting integration with biological and environmental monitoring	[27]

Psychological well-being will be quantified using the WHO-5 Well-Being Index, while psychosocial stress and occupational strain will be assessed with the Effort–Reward Imbalance (ERI) questionnaire. Work ability, considering job demands and health status, will be measured with the Work Ability Index (WAI).

To capture chemical exposures, participants will also complete the OSHA Chemical Exposure Questionnaire (CEQ), which documents frequency, intensity, and type of exposure to disinfectants and sterilants. Combined with biological and environmental monitoring, the CEQ allows an integrated assessment of the chemical component of the occupational exposome.

15. Daily Diaries

To capture real-life behaviors and occupational exposures, participants will complete daily diaries for 10–14 consecutive days, covering at least two night shifts for night-shift workers and a full work–rest cycle for day-shift workers. Diaries record detailed information on diet, sleep, and physical activity, providing contextual data on circadian disruption, lifestyle, and chemical risk.

15.1. Food Diary

Participants will log all food and beverages in real time, including timing, type, quantity, and estimated caloric content, allowing assessment of eating patterns relative to work shifts and potential interactions with chemical exposures.

15.2. Sleep Diary

Bedtime, wake time, sleep duration, awakenings, naps, and subjective sleep quality will be documented, contextualizing questionnaire scores and molecular biomarkers to evaluate circadian misalignment.

15.3. Physical Activity Diary

Type, duration, and intensity of both occupational (e.g., ward duties, handling disinfectants) and non-occupational activities (e.g., exercise, walking) will be recorded, complementing IPAQ data and linking activity patterns to chemical exposures.

Diary data will be integrated with biological and environmental monitoring to provide a comprehensive exposome-based assessment. Lifestyle patterns will be correlated with molecular biomarkers, including clock gene expression, circadian miRNAs, inflammatory cytokines, oxidative stress markers, and urinary metabolites of QACs, glutaraldehyde, and VOCs. Environmental sampling (surface and air) will be mapped to diary entries, contextualizing exposures relative to work tasks and shift schedules. This integrated approach enables identification of temporal relationships between NSW, chemical exposures, lifestyle behaviors, and early biological changes, supporting risk stratification, personalized interventions, and preventive strategies in HCW.

16. Work Environment Monitoring

A structured environmental monitoring program will quantify occupational chemical exposures across hospital departments. Surface wipe sampling will assess residues of QACs and glutaraldehyde, while airborne concentrations of VOCs, QAC aerosols, and glutaraldehyde vapors will be measured using area and personal samplers in high-risk zones (ICUs, emergency departments, ORs, CSSD).

Sampling schedules will consider peak activity, cleaning cycles, and night shifts to capture temporal variability. Samples will be analyzed using validated chromatographic and spectrophotometric methods. Environmental data will be integrated with individual urinary and blood biomarkers to characterize exposure–dose relationships, identify hotspots, and support risk stratification.

17. Clock Genes and Circadian miRNAs as Biomarkers of Breast Cancer Risk

Computational analyses, including our own, have identified clock genes and circadian miRNAs as potential early biomarkers of BC risk in night shift workers [5]. Differential expression patterns were observed in BC tissues and in healthy women with long-term night shift exposure. BHLHE40, CIART, CLOCK, PDPK1, TIMELESS were overexpressed, while HLF, NFIL3, NPAS3, PER1, PER3, SIM1, TEF were downregulated. Notably, PER1 and TEF downregulation with CLOCK upregulation correlated with increased BC susceptibility [5]. Twenty-six circadian-related miRNAs (e.g., miR-10a, miR-21, miR-107, miR-34) modulate these genes and are influenced by night shift exposure, affecting cell cycle, apoptosis, and DNA damage response. Assessment of these candidate

genes and miRNAs will be integrated with clinical, biochemical, and lifestyle data to explore predictive value and support early detection and personalized preventive interventions in female night shift HCW [5].

18. Integrated Assessment of Inflammatory, Metabolic, Hormonal, and Lipid Biomarkers

Night shift work induces circadian misalignment, metaflammation, and metabolic/hormonal dysregulation, which may be compounded by exposure to QACs, glutaraldehyde, and VOCs[13]. Serum and plasma will be analyzed for pro-inflammatory cytokines (TNF-α, IFN-γ, IL-6, IL-8, IL-18, IL-1β) using ELISA, reflecting early immune dysregulation.

Metabolic and hormonal biomarkers include fasting glucose, insulin (HOMA-IR), HbA1c, lipid profiles, liver enzymes (AST, ALT, γ-GT), renal function (creatinine, azotemia), vitamin D, cortisol, TSH, free T4, and total antioxidant status (TAS). Biomarkers of chemical exposure (urinary metabolites, 8-OHdG, DNA damage assays) will be integrated to assess internal dose and early effect responses.

These biochemical measures will be interpreted alongside anthropometric and body composition indices (BMI, waist circumference, FFMI, BCMI, SMI) to explore interactions between metabolic status, systemic inflammation, and chemical exposures. Correlative analyses will support early detection of subclinical alterations, refined risk stratification, and personalized preventive strategies.

19. Extracellular Vesicles as Biomarkers of Systemic Stress and Breast Cancer Risk

Extracellular vesicles (EVs) are lipid-bound nanoparticles carrying proteins, lipids, nucleic acids, and signaling molecules that reflect cellular status [28]. In night shift HCW, circadian disruption, sleep deprivation, chemical exposures, and psychosocial stress may alter EV release and composition, linking cellular stress to systemic inflammation, metabolic imbalance, and BC susceptibility [6,29].

Plasma-derived EVs will be isolated and analyzed for protein cargo, surface markers, and signaling molecules related to inflammation, metabolism, oxidative stress, and BC risk. Cargo may include cytokines (TNF-α, IL-6), adhesion molecules, growth factors, and miRNAs. EVs will be processed using standardized protocols, isolated by size-exclusion chromatography or ultracentrifugation, and characterized by NTA, electron microscopy, and immunoblotting for canonical markers (CD9, CD63, CD81).

20. Interventions and Follow-Up

After baseline assessment, participants will receive personalized recommendations targeting nutrition, sleep, physical activity, circadian alignment, and chemical exposure reduction. Advice will include Mediterranean-style diet timing, sleep hygiene strategies, tailored exercise plans, and occupational measures to minimize chemical contact and manage stress.

A 1-month follow-up will assess adherence, address barriers, and refine strategies. At 12 months, all clinical, biochemical, lifestyle, and exposure assessments will be repeated to evaluate changes in metabolic, inflammatory, circadian, and psychosocial outcomes.

21. Discussion and Conclusions

The exposome encompasses the totality of environmental exposures experienced from conception onward, and their cumulative effects on health (including chemical, physical, psychosocial, and lifestyle) [30]. Unlike traditional risk assessments that focus on single exposures, the exposome framework integrates multiple concurrent exposures and their interactions with genetic and physiological factors, providing a holistic perspective on health determinants [31]. This approach is particularly relevant in healthcare, where workers face complex combinations of chemical disinfectants, night shift work (NSW), psychosocial demands, and variable lifestyle factors. Focusing on female healthcare workers allows investigation of sex-specific physiological and hormonal influences, including potential implications for BC risk [32].

This study protocol embodies the exposome perspective by simultaneously evaluating chemical exposures (VOCs, QACs, glutaraldehyde), NSW, circadian disruption, lifestyle behaviors, and systemic biomarkers of metabolic, inflammatory, and hormonal status. Through comprehensive monitoring, including structured questionnaires, daily diaries, environmental measurements, and advanced biomarker analysis such as clock genes, circulating circadian miRNAs, and EVs, the protocol captures both external and internal components of the occupational exposome, enabling a multidimensional assessment of health risks in female HCW. By linking exposure data to molecular, physiological, and anthropometric endpoints, this approach allows the identification of early perturbations associated with systemic stress, metabolic dysregulation, and long-term disease risk, including BC.

Strengths of the protocol include its holistic design, integration of multi-level exposure data, use of objective biomarkers alongside self-reported information, and longitudinal follow-up, which allows evaluation of temporal exposure-response relationships and intervention effectiveness. Limitations include potential reporting bias, the snapshot nature of biomarker assessments, single-hospital generalizability, and participant burden. Restriction to female HCW enhances sex-specific insights but limits applicability to male workers.

Occupational physicians play a central role in implementation, interpreting data, advising on risk mitigation, and facilitating personalized interventions to optimize shift schedules, reduce chemical exposure, and protect worker health [33]. The exposome-oriented approach supports both individual-level preventive strategies (i.e., circadian-aligned nutrition, sleep hygiene, physical activity) and organizational policies, including optimized shift rotations, improved ventilation, and standardized chemical handling [34]. Integration of longitudinal health monitoring further strengthens the capacity to detect early pathophysiological changes associated with NSW and chemical exposures.

In conclusion, despite feasibility and compliance challenges, this protocol offers a model for integrative occupational risk assessment, with potential to help the implementation of clinical practice and public health strategies in hospital settings [35].

Supplementary Materials

The additional data and information can be downloaded at: https://media.sciltp.com/files/content/IJET-25120097-Author-Supplementary-File-V2.pdf. Table S1: Hospital departments vs. main chemical hazards, NSW burden, and monitoring priorities. Table S2: Health assessment variables. Table S3 Medical history variables. Table S4: Occupational history variables. Table S5: Blood parameters. Table S6: Urine parameters.

Author Contributions

S.V.: conceptualization, methodology, writing—original draft preparation, writing—reviewing and editing; M.C.: visualization, writing—original draft preparation, writing—reviewing and editing; C.F.: conceptualization, supervision, writing—original draft preparation, writing—reviewing and editing. All authors have read and agreed to the published version of the manuscript.

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Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

This manuscript describes a study protocol and does not report results from human participants; therefore, no datasets were generated or analyzed at this stage.

Acknowledgments

Not applicable.

Conflicts of Interest

The authors declare no conflict of interest.

Use of AI and AI-Assisted Technologies

During the preparation of this work, the author(s) used ChatGPT (OpenAI) to assist with language editing and clarity. After using this tool, the author(s) reviewed and edited the content as needed and take full responsibility for the content of the published article.

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