



Proceeding Paper

SEM Analysis of Red Blood Cell Morphology as a Biomarker in Agricultural and Industrial Environments: Initial Findings in Exposome Research [†]

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Abstract

Red blood cells (RBCs) are sensitive biomarkers of human health, influenced by urbanization and agricultural exposures. Using scanning electron microscopy (SEM) within an exposome framework, we examined RBC morphology in residents of an industrialized area of Thessaloniki, Greece, and in a rural population primarily exposed to agricultural stressors. Blood samples and questionnaires covering demographics, lifestyle, and environmental factors were statistically analyzed. SEM revealed moderate morphological alterations without significant differences between groups. Observed features were associated with longer residence duration and suboptimal nutrition, suggesting subclinical cellular stress. Integrating these findings into exposome research may clarify cumulative industrial and agricultural impacts on RBC morphology.

Keywords: scanning electron microscopy; optical microscopy; urban-industrial pollution; environmental health monitoring; subclinical biomarkers; red blood cells



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1. Introduction

Environmental exposures in contemporary societies involve complex mixtures of chemical and nonchemical stressors arising from industrial activities, urbanization, and agricultural practices [1]. These stressors often combine over long periods, contributing to cumulative biological effects that may remain subclinical for years. Conventional risk assessment typically evaluates single substances and isolated pathways, limiting interpretation under real-world, low-intensity mixed exposure conditions. The exposome framework addresses this gap by considering the totality of environmental exposures and related biological responses across the life course [2]. Exposome-oriented research therefore inte-

grates contextual data with early-effect phenotypic biomarkers to detect subtle biological perturbations preceding adverse outcomes [3,4].

Red blood cells (RBCs) are a relevant biological target, as they are continuously exposed to circulating stressors and reflect alterations in membrane stability, lipid organization, and cytoskeletal integrity [5]. Even minor morphological changes may indicate early cellular stress related to environmental or lifestyle factors [6]. Optical microscopy enables rapid assessment of RBC morphology, whereas scanning electron microscopy (SEM) provides high-resolution visualization of surface features and subtle structural alterations [7]. Their combined use supports qualitative characterization of RBC phenotypes within exposome research and facilitates exploratory assessment in complex exposure settings. Industrial and agricultural environments represent distinct exposure contexts involving airborne, waterborne, and occupational stressors [8]. This pilot study aimed to evaluate the feasibility of combining optical microscopy and SEM to qualitatively assess RBC morphology in adults living in industrial versus agricultural settings, and to explore whether environmental context is reflected in RBC phenotypic patterns within an exposome framework.

2. Materials and Methods

2.1. Study Design and Population

This pilot comparative sub-study was conducted within an exposome-oriented research framework in Greece [9]. Adult participants were recruited from two contrasting environmental settings: an industrially influenced area and a rural agricultural area. Both contexts may involve mixed exposure pathways; however, they were selected to represent distinct real-world exposure environments. A balanced sample was included (industrial $n = 10$; agricultural $n = 10$) to support the initial qualitative comparison. Recruitment targeted individuals from the general population, matched by age and sex, to minimize major confounding factors from acute hematological conditions. Written informed consent was obtained from all participants.

2.2. Questionnaire Data Collection

A structured interviewer-administered questionnaire collected contextual information relevant to exposome characterization, including sociodemographic characteristics, residential history, lifestyle factors (e.g., smoking), and exposure proxies such as occupation, time outdoors, and drinking water source. Data was used descriptively to support the interpretation of microscopy findings and explore potential contributors to variability, without inferential exposure modeling due to the pilot design.

2.3. Blood Sampling and Morphological Analysis of Red Blood Cells

Peripheral venous blood was collected by trained personnel under standard clinical conditions. Whole blood was collected in EDTA tubes to minimize coagulation and preserve cellular morphology [10]. Samples were handled under controlled conditions to reduce pre-analytical variability and were processed within an appropriate time window following collection to limit artifactual morphology changes. Aliquots of whole blood were used for the preparation of blood smears for optical microscopy and for SEM sample preparation. Standard biosafety procedures were applied throughout collection and processing [11].

Optical and Scanning Electron Microscopy

Blood smears were prepared according to standard hematological procedures using thin smears on glass slides. Following air-drying, smears were stained using a routine differential blood stain (May Grünwald Giemsa), enabling visualization of RBC morphology.

Slides were examined under standard magnification (e.g., $\times 40$ and $\times 100$ oil immersion where applicable). Optical microscopy assessment focused on overall RBC morphology, including general shape and size uniformity, and the presence of overt morphological abnormalities. Optical microscopy was performed using a MoticEasyScan optical microscopy scanner (Motic Hong Kong Limited, Hong Kong, China), located at the Laboratory of Histology-Embryology, School of Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece.

For SEM, samples underwent fixation, graded dehydration, and conductive coating prior to imaging. SEM analysis (accelerating voltage ~ 20 kV; magnification range as applicable) enabled high-resolution visualization of RBC surface morphology and subtle structural deviations from typical biconcave discocytes [12]. To reduce interpretational bias and enhance consistency, microscopy assessment was performed using a standardized qualitative approach. The findings were interpreted in combination with contextual information from the questionnaire, acknowledging that RBC morphology can be influenced by multiple interacting factors, including age, lifestyle patterns, and residence duration. As the present study represents an initial exploratory step, microscopy findings were not considered diagnostic but phenotypic indicators potentially relevant to early subclinical biological perturbations.

3. Results

3.1. Study Population Characteristics

A total of 20 adult participants were included in this pilot analysis ($n = 10$ industrial group, $n = 10$ agricultural group), with equal representation of females and males (50% each). Participants were aged 50–65 years (mean age 57.1 ± 4.8). Overall, 40% of participants were smokers, and 35% reported occupational chemical exposure. The mean duration of residence in the study area was 32.7 ± 14.6 years. Participants reported spending on average 3.3 ± 2.2 h/day outdoors (range: 1–10 h/day). Tap water was the primary household water source for most participants (70%), while 30% reported bottled water use. Stress-exposure proxy levels were distributed as low (40%), moderate (35%), and high (25%). Based on optical microscopy assessment, 45% of participants presented near baseline RBC morphology, whereas 55% showed mild-to-moderate morphological alterations (Table 1).

Table 1. Demographic and exposure profile of the study population ($n = 20$).

Variable	Categories/Range	Mean $\pm$ SD or %
Sex	10 Female, 10 Male	50%/50%
Age (years)	50–65	57.1 ± 4.8
Smoking status	8 smokers, 12 non-smokers	40%/60%
Years of residence (area)	10–60 years	32.7 ± 14.6
Time outdoors (h/day)	1–10	3.3 ± 2.2
Occupational chemical exposure	Yes: 7, No: 13	35%/65%
Water source	Tap: 14, Bottled: 6	70%/30%
Stress-exposure level (proxy)	Low: 8, Moderate: 7, High: 5	40%/35%/25%
RBC morphological status (Optical)	Normal: 9, Altered: 11	45%/55%

3.2. Optical Microscopy and SEM and RBC Morphology Scoring

Optical microscopy ($\times 40$) demonstrated heterogeneous RBC morphology across the study population, ranging from well-preserved discocytic profiles to mild and moderate deviations indicative of altered cell dispersion and shape stability. Based on the predefined

qualitative scoring scheme, 4/20 participants (20%) were classified as normal (score 0), 7/20 (35%) as mildly altered (score 1), and 9/20 (45%) as moderately altered (score 2) (Table 2).

Table 2. Optical microscopy (×40) RBC morphology scoring by group (n = 20).

Group	n	Normal (0)	Mild (1)	Moderate (2)
Industrial	10	1 (10%)	4 (40%)	5 (50%)
Agricultural	10	3 (30%)	3 (30%)	4 (40%)
Total	20	4 (20%)	7 (35%)	9 (45%)

Normal morphology (score 0) was defined by uniformly distributed biconcave discocytes with preserved central pallor and minimal artefactual clustering. Mild alterations (score 1) were characterized by low-grade aggregation, increased cell pairing or small clusters, and subtle shape variability, including early echinocyte or elliptocyte like features, without overt pathological forms. Moderate alterations (score 2) showed more pronounced non-classical aggregation patterns, reduced intercellular spacing, and combined shape deviations, consistent with subclinical membrane perturbation. When stratified by environmental setting, morphology scores showed no pronounced group separation. The industrial group presented a higher proportion of moderate alterations, whereas the agricultural group showed a more even distribution across categories (Table 2). These findings suggest comparable levels of qualitative RBC morphological variability across the two exposure contexts within this exploratory cohort. Representative optical microscopy and SEM images illustrating the observed RBC morphological patterns are shown in Figure 1.

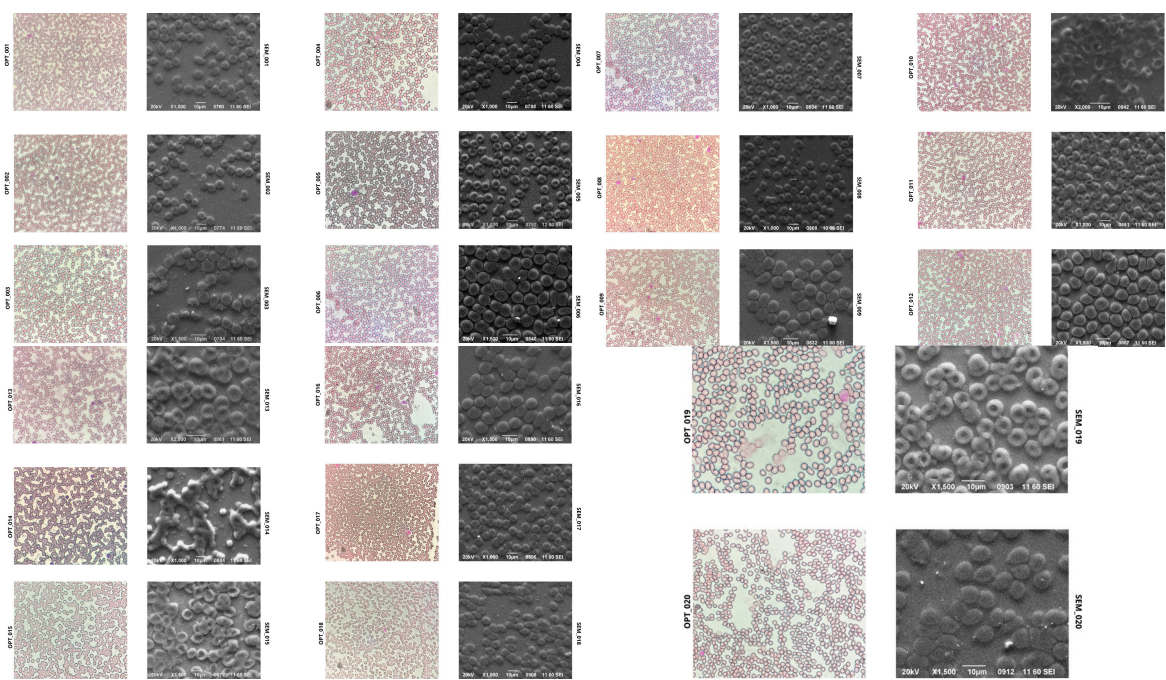


Figure 1. Optical microscopy (×40) and SEM of RBC morphology across participants (n = 20). Qualitative examination revealed three patterns that recur in addition to score-based classification: moderate shape instability phenotypes, aggregation-dominant profiles, and mixed forms that combine both characteristics. Severe pathological erythrocyte abnormalities, which are frequently associated with acute hematologic disease, were not observed.

Scanning electron microscopy (SEM) was applied in parallel with optical microscopy to further characterize RBC morphology at the ultrastructural level. For SEM, samples

underwent fixation, graded dehydration, and conductive coating prior to imaging. SEM analysis was performed using a JSM-6590LV scanning electron microscope (JEOL Ltd., Tokyo, Japan). SEM imaging enabled high-resolution visualization of RBC surface morphology and subtle structural deviations from typical biconcave discocytes [12]. SEM examination confirmed the predominance of discocyte morphology across samples while identifying subtle surface irregularities, early echinocytic transformations, and localized membrane deformations that were not consistently discernible by light microscopy. These ultrastructural observations refined the qualitative optical classification by improving the distinction between aggregation-related and shape instability phenotypes and by documenting mild-to-moderate membrane alterations across participants. Overall, the SEM findings supported the presence of low-grade, subclinical morphological variability without evidence of severe structural abnormalities.

4. Discussion

This pilot study used a combination of optical microscopy and scanning electron microscopy (SEM) to investigate red blood cell (RBC) morphology as a qualitative phenotypic indicator in an exposome oriented comparison of industrial and agricultural environments. Overall, RBC shape was essentially consistent across participants, with mild-to-moderate changes reported in both groups and no overt unusual phenotypes detected. These results imply that RBC morphological responses may mainly appear as modest and heterogeneous cellular changes rather than obvious group specific patterns under low-intensity, mixed real-world exposure conditions [13].

Although moderate alterations were slightly more frequent in participants residing in the industrial setting, substantial overlap in morphology scores between groups was observed. This probably reflects the multifactorial nature of environmental exposure in real-life situations, where individual characteristics like age, lifestyle factors, length of residence, occupational activity, and additional unknown variables influence biological responses in addition to environmental context [14]. Exposures rarely occur in isolation in exposome-based research, and low-grade cumulative stress might result in multiple and subclinical phenotypic responses that are difficult to quantify via small group comparison [15]. RBC shape is known to be sensitive to oxidative and metabolic changes that influence membrane stability and cell-cell interactions [16]. The modest echinocyte-like changes, aggregation tendencies, and surface abnormalities observed in this study appear more consistent with low-grade membrane stress than with overt hematologic disease. Importantly, SEM offered additional ultrastructural information by exposing membrane-level changes that were not consistently apparent under light microscopy, which assisted in the interpretation of minor subclinical variability across individuals [17].

The small sample size, qualitative approach, and lack of direct quantitative exposure assessments or biochemical indicators are some of this study's limitations. The objective of this pilot study was to investigate the viability of integrating microscopy-based phenotypic evaluation into an exposome framework rather than determining causal associations. Future research should increase the size of the cohort, use inter-observer validation and standardized morphological scoring, and combine the results of microscopy with environmental, clinical, and genomic data to increase the sensitivity of identifying biological indicators associated with exposure.

5. Conclusions

In this pilot study, optical microscopy demonstrated that more than half of the participants had mild-to-moderate RBC morphological variation without any severe dysmorphic forms. The overall distribution of morphology scores was similar for industrial and agri-

cultural contexts, indicating that a broad environmental context may not be sufficient to induce different group level morphological distinctions within small exploratory cohorts. Instead, the RBC shape appeared to represent low-grade and various cellular responses, which is consistent with ongoing, real-world exposure. Despite these limitations, the combination of optical microscopy and SEM provides a useful and informative method for capturing early, low-intensity cell responses to complicated environmental stresses. Within an exposome-oriented framework, qualitative RBC morphology may be utilized as a further phenotypic indicator for exploratory assessment and hypothesis development in studies that investigate cumulative environmental influences on human health.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in this study.

Data Availability Statement: Data is not publicly available due to ethical and privacy restrictions.

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