Ελληνικό Μεσογειακό Πανεπιστήμιο Κρήτης

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ΜΕΤΑΠΤΥΧΙΑΚΗ ΔΙΠΛΩΜΑΤΙΚΗ ΕΡΓΑΣΙΑ

Έκθεση σε περιβαλλοντικούς παράγοντες και υγεία: επιπτώσεις της έκθεσης σε Αφρικανική σκόνη στην υγεία και ο ρόλος των microRNAs ως διαγνωστικών μαρτύρων περιβαλλοντικής έκθεσης.

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Τριμελής επιτροπή:

Ευριδίκη Πατελάρου, Αναπληρώτρια Καθηγήτρια, Τμήμα Νοσηλευτικής, Ελληνικό Μεσογειακό Πανεπιστήμιο Κρήτης

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Ευχαριστίες

Θα ήθελα να εκφράσω τις ευχαριστίες μου στην επιβλέπουσα καθηγήτρια της μεταπτυχιακής μου εργασίας, κ. Πατελάρου Ευριδίκη, Αναπληρώτρια Καθηγήτρια του Τμήματος Νοσηλευτικής για την καθοδήγησή της, καθώς και για τον χρόνο που μου αφιέρωσε καθόλη τη διάρκεια της εκπόνησης και ως την ολοκλήρωση της προύσας διπλωματικής εργασίας.

Αισθάνομαι επίσης την ανάγκη να ευχαριστήσω θερμά την Επίκουρη Καθηγήτρια κ. Πατελάρου Αθηνά και το Λέκτωρα κ. Μιχαήλ Ζωγραφάκη για τη συμμετοχή τους ως μέλη της τριμελούς επιτροπής στη διαδικασία αξιολόγησης της συγκεκριμένης εργασίας.

Θα ήταν παράλειψη μου να μην αναφερθώ στους καθηγητές του συγκεκριμένου μεταπτυχιακού προγράμματος, τους οποίους ευχαριστώ από καρδιάς που σε όλη τη διάρκεια του κύκλου σπουδών στάθηκαν δίπλα μου, με απόλυτο στόχο τη μετάδοση των γνώσεών τους.

Τέλος, ένα μεγάλο ευχαριστώ οφείλω στην οικογένεια μου, που δίχως τη συμπαράσταση και τη βοήθειά τους όλο αυτό τον καιρό, η ολοκλήρωση των σπουδών μου δεν θα ήταν δυνατή.

Περιεχόμενα:

Σύνθεση των δύο δημοσιεύσεων

Ο Παγκόσμιος Οργανισμός Υγείας (Π.Ο.Υ.) θεωρεί τις επιπτώσεις της ατμοσφαιρικής ρύπανσης σαν μία από τις πιο πιεστικές προτεραιότητες για τις παγκόσμιες συνθήκες υγείας. Οι επιστήμονες συνδέουν την προερχόμενη από τη Σαχάρα σκόνη (ένα μετεωρολογικό φαινόμενο που μειώνει την ποιότητα του αέρα, καθώς εξαπλώνεται σε όλο τον πλανήτη) με συγκεκριμένες ασθένειες. Η Κρήτη συχνά πλήττεται από τη σκόνη της Σαχάρας, που αποτελεί κίνδυνο για την υγεία και προκαλεί ιδιαίτερη ανησυχία για τον νησιωτικό πληθυσμό και τις υπηρεσίες υγείας. Ως αερομεταφερόμενο ορυκτό, η σκόνη έχει τον σοβαρότερο αντίκτυπο στις υψηλότερες ηλικιακές ομάδες του πληθυσμού και ιδιαίτερα στα άτομα με αναπνευστικά προβλήματα, παρά το γεγονός ότι, κατά μέσο όρο, στο περιβάλλον οι συγκεντρώσεις σωματιδίων (PM) εμπίπτουν, ως επί το πλείστον, εντός των ορίων των διεθνών κατευθυντήριων γραμμών. Όλες οι περιβαλλοντικές εκθέσεις (συμπεριλαβανομένης και της Αφρικανικής σκόνης), στην πορεία της ζωής -ακόμα και από την προγεννητική περίοδο- επηρεάζουν την υγεία. Τα μικροRNAs (miRNAs) είναι ενδιαφέρουσες οντότητες εντός αυτής της έννοιας, μια και αναφέρονται ως δείκτες και πολλές φορές ως η αιτία για πολλές νόσους οφειλόμενες σε διάφορες περιβαλλοντικές εκθέσεις. Τα miRNAs είναι βραχείες ολιγονουκλεοτιδικές αλληλουχίες που μπορούν να αλληλεπιδράσουν με διάφορα mRNA στόχους.

Στο πρώτο μέρος της παρούσης εργασίας (Μέρος Α) παρουσιάζεται η σχετική δημοσίευση στο διεθνές Αγγλόφωνο Επιστημονικό Περιοδικό Biomarkers (Παράγοντας Επίπτωσης 1.73) της βιβλιογραφικής ανασκόπησης (Systematic Review) με τίτλο ‘MicroRNAs as biomarkers of harmful environmental and occupational exposures: a systematic review’. Έμφαση δίνεται στην τρέχουσα κατάσταση του πεδίου σχετικά με τις δυνατότητες χρήσης miRNAs ως βιοδεικτών για την έκθεση σε περιβαλλοντικούς παράγοντες -αυστηρά σε επίπεδο πληθυσμού και κλινικών μελετών. Στο δεύτερο μέρος της παρούσης εργασίας (Μέρος Β) παρουσιάζεται η σχετική δημοσίευση στο Διεθνές Αγγλόφωνο Περιοδικό International Journal of Occupational Medicine and Environmental Health (Παράγοντας Επίπτωσης 1.3) της βιβλιογραφικής ανασκόπησης (Systematic Review) με τίτλο ‘A systematic review of the health impact of Saharan dust exposure’, όπου παρουσιάζονται όλα τα τρέχοντα δεδομένα για την επίπτωση της Αφρικάνικής σκόνης στη Δημόσια υγεία.

Μέρος Α: Δημοσίευση με τίτλο 'MicroRNAs as biomarkers of harmful environmental and occupational exposures: a systematic review'

1 Γράμμα αποδοχής της σχετικής δημοσίευσης:

Από: Biomarkers <onbehalfof@manuscriptcentral.com>

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28-Jul-2019

Dear Dr Kotsyfakis:

Ref: MicroRNAs as biomarkers of harmful environmental and occupational exposures: a systematic review

Our referees have now considered your paper and have recommended publication in Biomarkers. We are pleased to accept your paper in its current form which will now be forwarded to the publisher for copy editing and typesetting. The reviewer comments are included at the bottom of this letter.

You will receive proofs for checking, and instructions for transfer of copyright in due course.

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Thank you for your contribution to Biomarkers and we look forward to receiving further submissions from you.

Sincerely,

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2 Περίληψη της σχετικής δημοσίευσης στην Αγγλική γλώσσα:

Abstract

Environmental exposure is a growing public health burden associated with several negative health effects. An estimated 4.2 million deaths occur each year from ambient air pollution alone. Biomarkers that reflect specific exposures have the potential to measure the real integrated internal dose from all routes of complex environmental exposure. MicroRNAs, small non-coding RNAs that regulate gene expression, have been studied as biomarkers in various diseases and have also shown potential as environmental exposure biomarkers. Here we review the available human epidemiological and experimental evidence of microRNA expression changes in response to specific environmental exposures including airborne particulate matter. In doing so, we establish that miRNA exposure biomarker development remains in its infancy and future studies will need to carefully consider biological and analytical “design rules” in order to facilitate clinical translation.

Keywords: Air pollution; biomarker; environmental exposure; heavy metals; microRNA; organic compounds; particulate matter

3 Περίληψη της σχετικής δημοσίευσης στην Ελληνική γλώσσα:

Η περιβαλλοντική έκθεση αποτελεί μια αυξανόμενη επιβάρυνση για την υγεία που συνδέεται με πολλές αρνητικές επιπτώσεις σε αυτήν. Υπολογίζεται ότι 4.2 εκατομμύρια θάνατοι συμβαίνουν κάθε χρόνο λόγω της ρύπανσης του ατμοσφαιρικού αέρα. Οι βιοδείκτες που αντανακλούν συγκεκριμένες εκθέσεις έχουν τη δυνατότητα να μετρήσουν την πραγματική και συνολική δόση/έκθεση σε σύνθετες (πολλαπλές) περιβαλλοντικές εκθέσεις. ΜικροRNAs, μικρά μη κωδικοποιητικά RNAs που ρυθμίζουν την γονιδιακή έκφραση, έχουν μελετηθεί ως βιοδείκτες σε διάφορες ασθένειες και έχουν επίσης προταθεί ως βιοδείκτες έκθεσης στο περιβάλλον. Εδώ εξετάζουμε τις διαθέσιμες επιδημιολογικές και πειραματικές ενδείξεις μεταβολών της έκφρασης του μικροRNA σε ανθρώπους ως απόκριση σε συγκεκριμένες περιβαλλοντικές εκθέσεις που περιλαμβάνουν και τα αιωρούμενα σωματίδια. Διαπιστώνουμε ότι η χρήση του μικροRNA ως βιοδείκτη περιβαλλοντικής έκθεσης παραμένει σε νηπιακό στάδιο και οι μελλοντικές μελέτες θα πρέπει να εξετάσουν προσεκτικά βιολογικούς και αναλυτικούς «κανόνες πειραματικού σχεδιασμού» για να διευκολύνουν την κλινική μετάφραση.

Λέξεις-κλειδιά: βιοδείκτης, περιβαλλοντική έκθεση, βαριά μέταλλα, μικροRNA, οργανικές ενώσεις, αιωρούμενα σωματίδια

4 Κείμενο της σχετικής δημοσίευσης στην Αγγλική γλώσσα:

MicroRNAs as biomarkers of harmful environmental and occupational exposures: a systematic review

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Clinical significance

A validated environmental exposure biomarker could have clinical, research, public health, and policy benefits. Current research in miRNA exposure biomarker development is in the very earliest phase of discovery. To make progress in the field, clinical researchers will need to address (i) the inherent complexity of the system being studied, and (ii) best practice for analytical methods for biomarker discovery and validation. There is a strong biological rationale for using miRNAs as exposure biomarkers since they are easy to assay and participate in exposure-related disease, but future studies will need to carefully consider these "design rules" for clinical translation.

Introduction

Exposure to environmental agents is a global public health concern associated with a wide range of adverse health outcomes through gene-environment interactions. According to the World Health Organization (WHO), ambient air pollution contributes to 4.2 million deaths per year, household exposures such as smoke and cooking fumes contribute to 3.8 million deaths per year, and 91% of the world's population resides in regions where air quality does not meet WHO recommendations (World Health Organization, 2016).

Environmental exposures are usually chemical mixtures with uncertain effects in

complex biological systems, so the risk they pose to human health is difficult to predict. Efforts are underway to develop biomarkers that reflect specific environmental exposures (exposure biomarkers), link these exposures to disease states (disease biomarkers), and predict disease risk (susceptibility biomarkers) (DeBord *et al.*, 2015). Given the complexity of human environmental exposures – sometimes referred to as the "exposome" (Vrijheid, 2014) – and the difficulty in measuring them in their totality, the advantage of biomonitoring through exposure biomarkers is that they have the potential to measure the real integrated internal dose from all routes of exposure.

A biomarker of exposure is a chemical, its metabolite, or the product of an interaction between a chemical and some target molecule or cell that is measured in the human body. There is growing evidence indicating that environmental exposures may alter gene expression, in part through the altered expression of specific microRNAs (miRNAs). Accordingly, miRNAs represent novel potential biomarkers of environmental exposure (Vrijens *et al.*, 2015). miRNAs are small, non-coding RNAs that regulate gene expression, so can provide a link between gene expression alterations from environmental agents and various disease states (Vrijens *et al.*, 2015). Several features make miRNAs potentially good biomarkers including their molecular stability, tissue specificity, and suitability for testing using several laboratory methodologies (Valencia-Quintana *et al.*, 2014). The biogenesis of miRNAs is depicted in **Figure 1** and described in detail by Macfarlane and Murphy (2010).

Exposure, disease, and susceptibility biomarkers all seek to answer different questions along the continuum from exposure to environmental illness. In this short review we focus on the first of these, exposure biomarkers, since defining the extent and impact of exposure underpins gaining a better understanding of environmental illness, mindful that there is no harm without exposure but also that exposure does not necessarily equate to harm. We systematically review all current human epidemiological and experimental data evidencing alterations in miRNA expression in response to defined environmental exposures in order to explore the current state of development and applicability of miRNA exposure biomarkers to human biomonitoring.

Search strategy

To identify studies related to this topic, we followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (http://prisma-statement.org/).We

searched PubMed using the term "miRNA AND environmental exposure AND biomarker". Studies selected for this review (after removing duplicates) were limited to those conducted on humans and written in English. The last search took place on June 3rd 2019.

Results

Seventeen articles described human epidemiological or experimental studies of miRNA expression in response to specific environmental exposures with the purpose of discovering miRNA exposure biomarkers (**Figure 2**). Of these, five related to ambient particulate matter (PM) in air pollution (**Table 1**), while twelve specifically related to exposures to heavy metals or occupational hazards (**Table 2**). All studies were cross-sectional, cohort, or crossover experimental studies. While the majority of studies detected global or a large panel of miRNAs using microarrays or sequencing platforms, a few studies adopted a candidate approach, quantifying miRNA expression using quantitative PCR (qPCR). Hence, there was no methodological standardisation across studies.

Ambient particulate matter exposure and miRNA expression

According to the WHO, air pollution is the most significant environmental threat to health. In 2012, one out of nine deaths were associated with air pollution, with approximately three million of those deaths attributed to ambient, or outdoor, pollution. Ambient particulate matter (PM) is a type of air pollutant comprised of solid particles and liquid droplets from the air, such as dust or soot. Both long- and short-term exposure to PM can be health-damaging, with particles less than 10 micrometres (PM_{10}) in diameter being the most dangerous (World Health Organization, 2016).

The five studies specifically examining miRNAs as biomarkers of human exposure to PM, either under controlled or city conditions, are summarised in **Table 1**. All studies identified several miRNAs associated with exposure to PM. For example, a pilot study of older individuals conducted by Rodosthenous *et al.* (2016a) demonstrated upregulation of miRNAs found in extracellular vesicles (evmiRNA) in response to exposure to PM less than 2.5 micrometers in diameter ($PM_{2.5}$). In a subsequent analysis, the gene targets of these evmiRNAs were involved in pathways related to cardiovascular disease, oxidative stress, inflammation, and atherosclerosis, thereby providing a putative mechanistic link between the exposure and subsequent adverse

health sequelae (Rodosthenous *et al.*, 2016a). Similarly, an experimental crossover study of individuals exposed to traffic-related air pollution identified fifty-four circulating miRNAs associated with exposure to various components of traffic air pollution, including PM_{10}, $PM_{2.5}$, black carbon, ultrafine particles, and nitrogen dioxide (Krauskopf *et al.*, 2018). Again, further bioinformatics analyses suggested that many of these miRNA target genes may be associated with cardiovascular, respiratory, renal, and neurologic disease processes.

The other studies reported similar associations of miRNAs and PM exposure. Conspicuously, however, none of the miRNAs implicated in individual studies were reproduced across different studies and, in the one study that applied the same methodologies to different cohorts (individuals exposed to city pollution in London and Barcelona), there was only limited overlap in miRNA hits between the two cohorts (Espin-Perez *et al.*, 2018). The reasons for this all likely to be multifactorial but include heterogeneity in tested exposures and tested populations; experimental differences between studies; different miRNA detection methodologies and limits of detection; intrinsic biological variability; small amplitude of effect; or lack of biological relevance of the miRNA to the exposure. It is therefore not possible to conclude that there are currently any validated miRNA biomarkers of exposure to PM.

Heavy metal or occupational hazard exposure and miRNA expression

Twelve studies have examined miRNA expression in humans exposed to a variety of heavy metals or occupational hazards including arsenic, polyaromatic cyclic hydrocarbons (PAHs), pesticides, vinyl chloride from plastic manufacturing, or volatile organic compounds (VOCs) such as toluene and benzene. The results of these studies are summarized in **Table 2**.

For example, volatile organic compounds (VOCs) may react with sunlight and other pollutants to form ground-level ozone, which has several negative health effects including cardiovascular disease, respiratory illness, and cancer. Benzene, a type of VOC and common air pollutant, is a confirmed carcinogen that may increase the risk of lung cancer (Liu *et al.*, 2016). Often used as industrial solvents, benzene and other VOCs represent significant occupational hazards and therefore an important public health problem (World Health Organization, 2016). Bai *et al.* (2014) identified a number of miRNAs as possible indicators of benzene exposure, with six upregulated miRNAs and seven downregulated miRNAs found in chronic benzene poisoning individuals compared to healthy controls.

The role of both metals and polycyclic aromatic hydrocarbons (PAHs) on the expression

of ten candidate miRNAs (let-7b-5p, miR-126-3p, miR-142-5p, miR-150-5p, miR-16-5p, miR-24-3p, miR-27a-3p, miR-28-5p, miR-320b, and miR-451a) was explored in a sample of 360 healthy male coke oven workers (Deng *et al.*, 2019). miRNA expression was negatively associated with aluminium, antinomy, lead, and titanium exposure, whereas it was positively associated with molybdenum and tin exposure. Antagonistic interactions between antinomy and monohydroxy-PAH and synergic interactions between plasma benzo[a]pyrene-r-7,t-9,t-9,c-10-tetrahdrotetrol-albumin were found. Several of the miRNAs, including let-7b-50, miR-126-3p, miR-16-5p, and miR-320b, were associated with genetic damage. These findings suggest possible mechanisms underlying the interaction between metal and PAHs and adverse health effects (Deng *et al.*, 2019).

The exposures in the summarised studies were heterogeneous, so it is perhaps unsurprising that the miRNAs detected were also different in response to different agents. Two studies of either two or three candidate miRNAs detected by qPCR in adults and children exposed to high arsenic levels in drinking water in Mexico both associated miR-126 with urinary arsenic concentrations (Perez-Vazquez *et al.*, 2017, Ruiz-Vera *et al.*, 2019). However, in two different studies of the same cohort of steel workers exposed to metal-rich PM using different methodological approaches (qPCR for miRNA candidates in (Bollati *et al.*, 2010) and miRNA microarrays as a discovery approach in (Motta *et al.*, 2013)), the associated miRNAs differed between the studies. This latter observation elegantly exposes how adopting different methodological approaches, even with the same samples from the same study participants, can produce different results.

Discussion

miRNAs are present throughout the genome, regulating gene expression in response to several cues. The summarised literature shows that differential miRNA expression can be detected in association with several different environmental exposures and that the gene targets of these miRNAs provide plausible mechanistic links between the exposure and disease pathogenesis. However, even when the exposure was the same or similar in different studies, the miRNAs detected were different. Overall, this review of the published literature shows that there are currently no miRNAs that can be regarded as reliable exposure biomarkers. Furthermore, although the *in silico* analyses of the biological pathways and processes impacted by the gene

targets of the candidate exposure biomarker miRNAs provide plausible links with disease pathogenesis and therefore potentially some insights into how the exposure modifies disease risk, no study experimentally or functionally validated the biological sequelae of miRNA dysregulation in response to the specified environmental risk factors.

Overall, the quality of the reviewed studies was good, with many being prospective, randomised, and/or adopting well-considered crossover designs and appropriate miRNA detection assays; some studies even provided some analytical validation by using qPCR to validate the miRNA candidates detected using high-throughput screening approaches such as microarrays. Therefore, it is possible that some of the detected miRNAs represent true candidate exposure biomarkers. A validated exposure biomarker could have clinical, research, public health, and policy benefits, and additionally a functional biomarker that is also disease causing or modifying, such as potentially the case with miRNAs (compared, for instance, with biomonitoring of the exposure itself such as urine arsenic levels), might have additional benefit as a simultaneous biomarker of disease or susceptibility.

Therefore, while study of miRNAs as exposure biomarkers is useful, current efforts in miRNA exposure biomarker development can only be regarded as being in the very earliest phase of discovery and in their infancy. Some of the reasons for this disconnect between discovery and utility have been summarised above: heterogeneity in exposures and populations; experimental variability; different methodologies and limits of detection; intrinsic biological variability; small amplitude of effect; or lack of biological relevance of miRNAs.

In order to make progress in the field, we suggest that clinical researchers will need to address two main issues. First, the inherent complexity of the system being studied: study designs will need to take into consideration the heterogeneous and multidimensional nature of both exposures and responses, perhaps by adopting new systems biology or computational approaches to deconvolute the relationship between exposome and genome (DeBord *et al.*, 2015). Second will be to adhere to best practice for analytical methods for biomarker discovery and validation, including ensuring precision, eliminating bias, and ensuring analytical specificity during analytical validation; evaluating assay performance in different laboratories and in different populations during clinical validation; and applying the test in prospective randomised studies when establishing clinical utility (Holland, 2016). Therefore, while there is a strong biological rationale for using miRNAs as exposure biomarkers since they have potential as both

robust assays and as participants in environmental exposure-related disease, future studies will need to carefully consider these “design rules” in order to facilitate clinical translation.

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Data sharing statement: None.

5 Πίνακες και Εικόνες της σχετικής δημοσίευσης:

Table 1. Studies discovering biomarkers of environmental exposure to air pollution

Reference	(Krauskopf *et al.*, 2018)	(Espin-Perez *et al.*, 2018)	(Rodosthenous *et al.*, 2016b)	(Fossati *et al.*, 2014)	(Yamamoto *et al.*, 2013)
Population	n=24 subjects, n=12 male and n=12 female non-smokers	Cohort 1, n=59 (London study, EXPOsOMICS project) Cohort 2, n=30 (Barcelona, Spain)	n=22 healthy individuals	n=153 elderly men	n=13 individual with asthma
Exposure/ detected compound(s)	Exposure to traffic in central London for 2 h; crossover study	Exposure to city pollution in London and Barcelona	$PM_{2.5}$ exposures	Ambient particles	Diesel exhaust
Study design	Crossover experimental	Crossover experimental	Cross-sectional	Cohort	Crossover experimental
Implicated miRNA(s)	miR-133a-3p; miR-193b-3p; miR-1224-5p; miR-433-3p; miR-145-5p; miR-27a-5p; miR-580-3p; miR-3127-5p; miR-6716-3p	miR-197-3p, miR-29a-3p, miR-15a-5p, miR-16-5p and miR-92a-3p	miR-126-3p, miR-19b-3p, miR-93-5p, miR-223-3p, miR-142-3p	miR-1, miR-1, miR-126, miR-135a, miR-146a, miR-155, miR-21, miR-222, miR-9	miR-21, miR-30e, miR-215, miR-144
miRNA detection technology	Small RNA-seq	miRNA microarrays	NanoString sequencing	qPCR	NanoString sequencing
Results	54 circulating miRNAs to be dose- and pollutant species-dependently associated with PM_{10}, $PM_{2.5}$, black carbon, ultrafine particles and NO_2	Compound-specific miRNA expression differences in exposed cohorts	Association between long-term ambient $PM_{2.5}$ levels and increased levels of extracellular vesicle miRNAs circulating in serum	Exposure to ambient particles could cause a downregulation of miRNAs involved in processes related to PM exposure	Expression of miR-21, miR-30e, miR-215, and miR-144 was significantly associated with diesel exhaust
Notes	miRNA gene targets associated with breast cancer, cardiovascular disease, respiratory disease, neurodegenerative disease, and kidney disease	Limited overlap in hits between the two cohorts	miRNA gene targets associated with cardiovascular disease-related pathways		

Abbreviations: PM=particulate matter; qPCR=quantitative real-time polymerase chain reaction

Table 2. Studies discovering biomarkers of environmental exposure to heavy metals and other occupational hazards

Reference	Population	Exposure/ detected compound(s)	Study design	Implicated miRNA(s)	miRNA detection technology	Results	Notes
(Ruiz-Vera *et al.*, 2019)	n=105 women, Mexico	Inorganic arsenic in drinking water	Cross-sectional	miR-155, miR-126	3 candidate miRNAs by qPCR	miR-155 and miR-126 levels associated with urinary arsenic levels	
(Deng *et al.*, 2019)	n=360 healthy male coke oven workers; control group (n = 238) and exposed group (n = 122)	Metals, PAHs, combined pollutants	Case-control	let-7b-5p, miR-126-3p, miR-142-5p, miR-150-5p, miR-16-5p, miR-24-3p, miR-27a-3p, miR-28-5p, miR-320b, and miR-451a	10 candidate miRNAs qPCR	miRNA expression negatively associated with aluminium, antimony, lead, and titanium, and positively associated with molybdenum and tin	Let-7b-5p, miR-126-3p, miR-16-5p, and miR-320b also associated with double-strand breaks in lymphocytes
(Perez-Vazquez *et al.*, 2017)	n=73 children (6-12 years)	Inorganic arsenic in drinking water	Cross-sectional	miR-126	2 candidate miRNAs by qPCR	Significant negative association ($p < 0.05$) between urinary arsenic concentrations and plasma miR-126 levels	
(Krauskopf *et al.*, 2017)	n=6 healthy subjects	Six PCB congeners, DDE (a common DDT metabolite), and HCB	Cross-sectional	miR-193a-3p, miR-152, miR-31-5p, miR-532-3p, miR-324-3p, miR-320d, miR-320a, miR-486-5p, miR-34a-5p, miR-331-3p, miR-21-5p, miR-501-3p	miRNA microarrays	93 miRNAs significantly associated with POP exposure	
(Feng *et al.*, 2017)	n=12; n=6 low and n=6 high VCM-exposed workers	Vinyl chloride monomer	Case-control	miR-222–3p, miR-146a-5p and miR-151a-5p, miR-22–3p	miRNA microarrays	miR-222–3p, miR-146a-5p and miR-151a-5p were downregulated, while miR-22–3p was upregulated in VCM-exposed group	
(Woeller *et al.*, 2016)	n=400; n=200 each case and control	Open burn pits in war zones; polychlorinated dibenzo-p-dioxins/dibenzofurans	Case-control	miR-145-5p, miR-17-5p	miRNA PCR arrays	Six miRNAs were significantly different in the case group after deployment and 17 miRNAs were significantly different between the case and control groups after case deployment to sites with open burn pit	
(Weldon *et al.*, 2016)	n=27 parent/child farmworker/non-farmworker pairs (n=16FW/n=11NFW)	Organophosphate (OP) pesticides	Case-control	miR-223, miR-518d-3p, miR-517b, miR-597, miR-133b	miRNA PCR arrays	Six miRNAs were observed to be positively associated with farmworkers status during the post-harvest season. Expression of five of these miRNA trended towards a positive dose response relationship with organophosphate pesticide metabolites in farmworkers	Urine miRNA detection
(Huang *et al.*, 2016)	n=365 healthy male coke oven workers; control group (n=241) and exposed group (n=124)	Urinary PAH metabolites	Case-control	miR-24-3p, miR-27a-3p, miR-142-5p, miR-320b	6 candidate miRNAs qPCR	miR-24-3p, miR-27a-3p, and miR-320b were significantly interacted with multiple PAH metabolites	

(Song and Ryu, 2015)	n=50, n=22 control and n=28 exposed	Toluene, xylene, and ethylbenzene	Case-control	N/A	miRNA microarrays	467 miRNAs for toluene, 211 miRNAs for xylene, 713 and 695 miRNAs for ethylbenzene that exactly annotated each exposure 714 group and unexposed control group, with high accuracy	
(Bai *et al.*, 2014)	n=10, n=7 cases and n=3 controls	Chronic benzine poisoning	Case-control	miR-34a, miR-205, miR-10b, let-7d, miR-185, miR-423-5p-2, miR-133a, miR-543, hsa-miR-130a, miR-27b, miR-223, miR-142-5p, miR-320b	miRNA microarrays	6 up-regulated miRNAs and 7 down-regulated miRNAs found in chronic benzene poisoning group compared to healthy controls	
(Motta *et al.*, 2013)	n=63 foundry workers with well-characterized exposure to metal-rich PM	Metal-rich particulate matter	Cohort	miR-421, miR-146a, miR-29a, let-7g	miRNA microarrays	Four miRNAs were differentially expressed in postexposure compared with baseline samples	
(Bollati *et al.*, 2010)	n=63 steel workers	Metal-rich particulate matter	Cohort	miR-222, miR-21	qPCR	Expression of miR-222 and miR-21 significantly increased in postexposure samples. In postexposure samples, miR-222 expression was positively correlated with lead exposure and miR-21 expression was associated with blood 8-hydroxyguanine	

Abbreviations:DDE=dichlorodiphenyldichloroethylene;DDT=dichlorodiphenyltrichloroethane; FW=farmworker; HCB=hexachlorobenzene; PAH=polyaromatic cyclic hydrocarbon; PCB=polychlorinated biphenyl; PM=particulate matter; POP=persistent organic pollutant; qPCR=quantitative real-time polymerase chain reaction; VCM=vinyl chloride monomer

Figure 1. MicroRNA biogenesis and mechanism of action. Depicted is the process of miRNA formation, starting from the miRNA gene and resulting in mature miRNA. Note that the mature miRNA silences genes either through mRNA cleavage or translation repression. Abbreviation: RISC: RNA-induced silencing complex. This figure and information were adapted from Macfarlane and Murphy (2010).

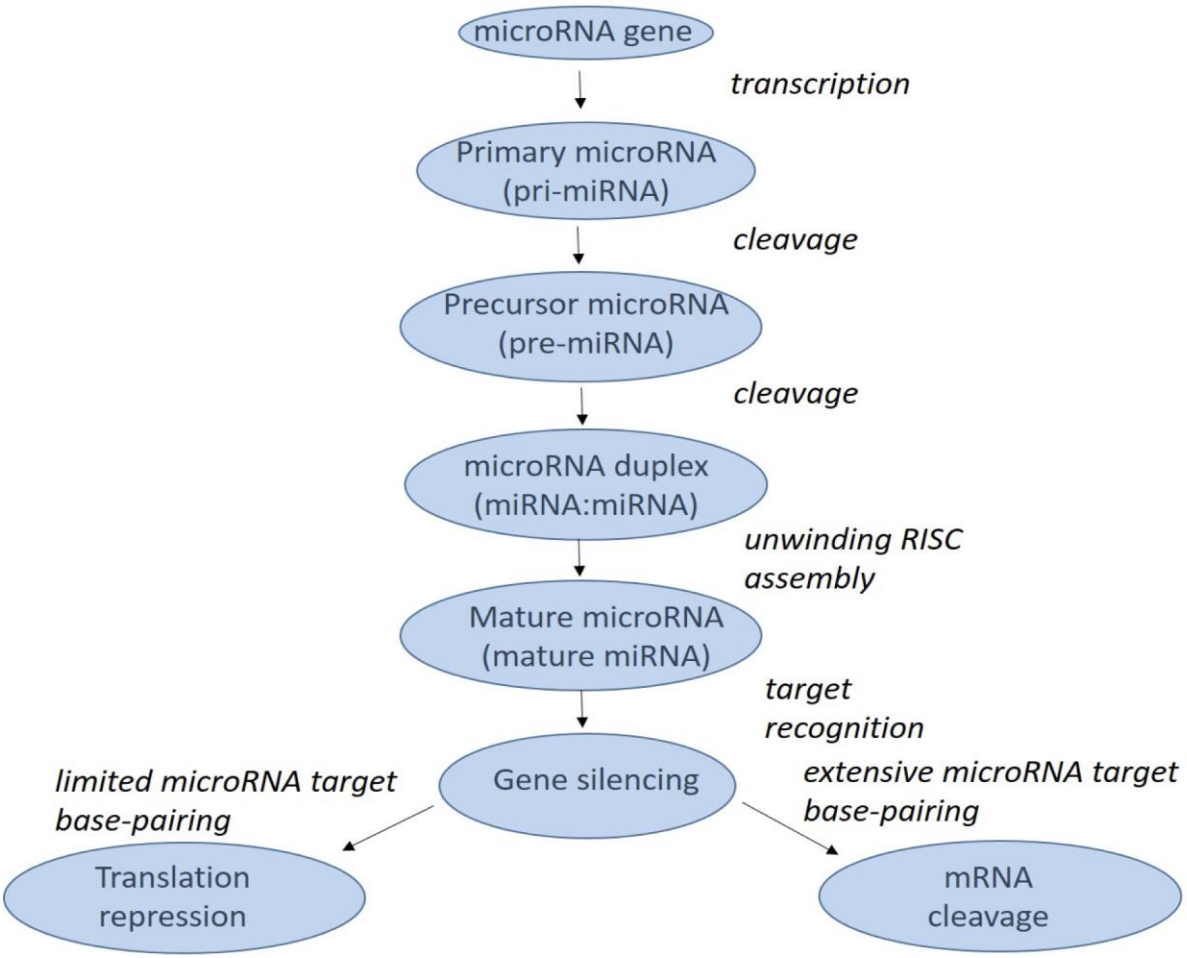
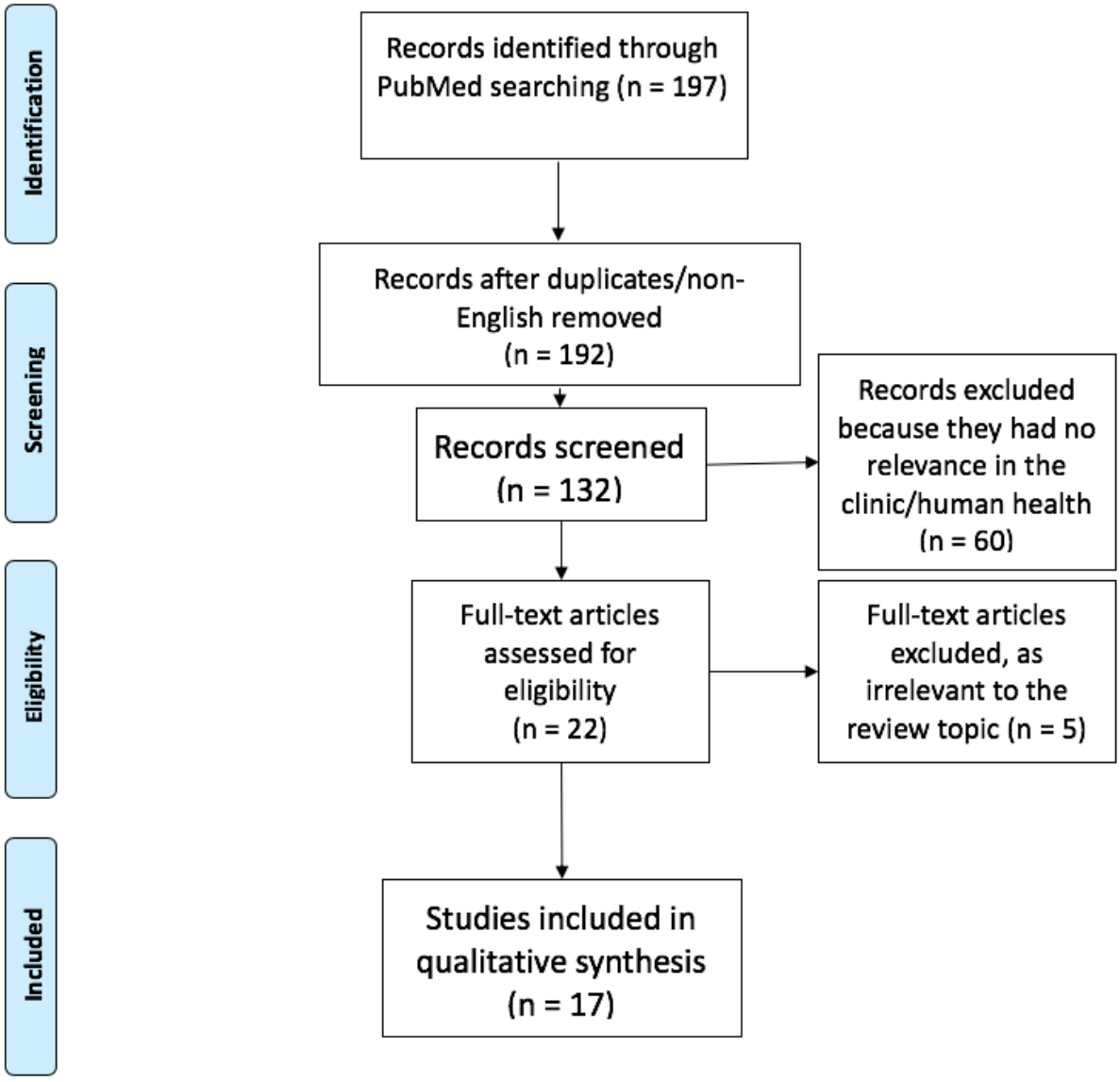


Figure 2. PRISMA flow diagram for the literature search performed in this review.



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Μέρος Β: Δημοσίευση με τίτλο 'A systematic review of the health impact of Saharan dust exposure'

1 Γράμμα αποδοχής της σχετικής δημοσίευσης:

Από: International Journal of Occupational Medicine and Environmental Health <kontakt@editorialsystem.com>

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A systematic review of the health impact of Saharan dust exposure

Dear Michail Kotsyfakis,

I am pleased to inform you that your manuscript entitled A systematic review of the health impact of Saharan dust exposure has been finally accepted for publication in the bimonthly "International Journal of Occupational Medicine and Environmental Health".

Thank you for the payment and the Transfer of Copyrights Agreement that has been sent.We will notify you of the subsequent stages of the publishing process.

Thank you for submission of your article to our journal. You are more than welcome to send your subsequent papers.

Yours sincerely,

Prof. Konrad Rydzyński

Editor-in-Chief

"International Journal of Occupational Medicine and Environmental Health"

2 Περίληψη της σχετικής δημοσίευσης στην Αγγλική γλώσσα:

Abstract

Air pollution is a high priority global health concern. The health damaging effects of ambient particulate matter (PM), a component of air pollution, are extensively documented, with 1.4% of deaths worldwide resulting from exposure to particulate matter. A growing body of evidence suggests that mineral dust, found in particulate matter, may contribute to some of these deleterious health impacts. Approximately half of atmospheric mineral dust originates from the Saharan desert. This systematic but concise review summarizes the findings from recent literature exploring the adverse health effects of Saharan dust particles worldwide. We show that (i) PM contributes to all-cause and causespecific mortality and morbidity; (ii) PM arising from Saharan dust contributes to excess all-cause and cause-specific mortality and morbidity; and (iii) larger particle sizes may be more harmful than smaller particle sizes. However, there remain many questions regarding their effects on vulnerable patient populations, underlying mechanisms of action, and regional variations in both environmental and health effects. This review highlights the urgent need for continued and deeper analyses of this emerging public health issue.

Keywords: air pollution, particulate matter, public health, dust, Africa, Northern

3 Περίληψη της σχετικής δημοσίευσης στην Ελληνική γλώσσα:

Η ατμοσφαιρική ρύπανση αποτελεί παγκόσμιο πρόβλημα υγείας. Οι επιβλαβείς για την υγεία επιπτώσεις των αιωρούμενων σωματιδίων του περιβάλλοντος (PM), ενός συστατικού της ατμοσφαιρικής ρύπανσης, τεκμηριώνονται εκτενώς, με 1,4% των θανάτων παγκοσμίως να είναι αποτέλεσμα της έκθεσης σε σωματίδια. Ένα αυξανόμενο σύνολο στοιχείων υποδηλώνει ότι η σκόνη ορυκτών, η οποία απαντάται σε σωματίδια, μπορεί να συμβάλει σε ορισμένες από αυτές τις επιζήμιες επιπτώσεις στην υγεία. Περίπου το ήμισυ της ατμοσφαιρικής ορυκτής σκόνης προέρχεται από την έρημο της Σαχάρας. Αυτή η συστηματική, αλλά συνοπτική ανασκόπηση βιβλιογραφίας συνοψίζει τα ευρήματα από την πρόσφατη βιβλιογραφία που διερευνά σε όλο τον κόσμο τις αρνητικές συνέπειες για την υγεία των σωματιδίων σκόνης της Σαχάρας. Δείχνουμε ότι (i) τα PM αποτελούν τη γενική αιτία και προκαλούν ειδική θνησιμότητα και νοσηρότητα. (ii) τα PM που προέρχονται από τη σκόνη της Σαχάρας συμβάλλουν στην αύξηση της θνησιμότητας και της νοσηρότητας, και (iii) τα μεγαλύτερα μεγέθη σωματιδίων μπορεί να είναι πιο επιβλαβή από τα μικρότερα μεγέθη σωματιδίων. Ωστόσο, εξακολουθούν να υπάρχουν πολλά ερωτήματα σχετικά με τις επιπτώσεις τους στους ευάλωτους πληθυσμούς των ασθενών, τους βασικούς μηχανισμούς δράσης και τις κατά τόπους διαφορές, όσον αφορά τόσο τις επιπτώσεις στο περιβάλλον, όσο και στην υγεία. Αυτή η ανασκόπηση βιβλιογραφίας υπογραμμίζει την επείγουσα ανάγκη για συνεχείς και βαθύτερες αναλύσεις αυτού του αναδυόμενου ζητήματος δημόσιας υγείας.

Λέξεις κλειδιά: ατμοσφαιρική ρύπανση, σωματίδια, δημόσια υγεία, σκόνη, Αφρική, Βόρεια

4 Κείμενο της σχετικής δημοσίευσης στην Αγγλική γλώσσα:

A systematic review of the health impact of Saharan dust exposure

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Running Title: Saharan dust exposure and human health

Introduction

The World Health Organization (WHO) regards air pollution as a top global health priority [1]. The adverse effects of particulate matter (PM), a component of air pollution, on human health are well documented. The WHO estimates that 1.4% of all deaths worldwide result from exposure to PM [2]. One of the components of PM, atmospheric mineral dust, has recently attracted attention since it may be responsible for some of the hazardous effects of PM [3].

The main source of atmospheric mineral dust is from the desert, with approximately half originating from the Sahara Desert [3], although dust also spreads from other regions including the Arabian Peninsula, Central Asia, China, Australia, America, and South Africa. Each year, one to three gigatons of dust are emitted from these regions [4]. Sand and dust storms frequently occur in semi-arid and arid climates. Thunderstorms and cyclones produce strong pressure gradients that increase the wind speed. The wind then lifts and disperses large amounts of sand and dust from the soil into the atmosphere. This dust can spread several thousand kilometers from its origin. Sometimes, precipitation clears atmospheric dust, leading to wet rather than dry deposits of dispersed dust. Therefore, climatic conditions play a significant role in desert dust

movement. Since vegetation can protect the ground from erosion during storms, droughts and comparable environmental conditions may contribute to the development of dust storms [4].

The Sahara Desert in disperses dust worldwide, with 12% travelling north to Europe, 28% west to America, and 60% south to the Gulf of Guinea [3]. Saharan dust then contributes to PM levels exceeding threshold limits established by the European Union (EU) and WHO [3]. Almost four million tons of desert dust from the Sahara are transported to Mediterranean regions, leading to high PM levels. Notably, during Saharan dust events, the mineral dust concentrations in PM increase by 35% and PM concentrations are increased in general. One study showed that of six exceedances of EU threshold limits in a six-month period, five occurred during Saharan dust days [5].

Due to the potential health impact of its dispersal, the deleterious effect of atmospheric dust is now emerging as a global health concern. As a result, there has been increasing interest in the role of Saharan dust dispersion on health over the last two decades [2]. Several theories on how Saharan dust impacts human health have been proposed. Given that Saharan dust is a component of PM it is respirable, so could potentially increase the risk of respiratory and related illnesses and consequent related cause-specific and total mortality. Additionally, Saharan dust dispersion has been linked to the transport of various micro-organisms, so may also cause infectious diseases [2, 3].

In this short review, we summarize the findings from recent literature exploring the association between Saharan dust particles and human health to collate the available evidence and establish areas of research that require further effort in order to better understand and eventually tackle this important domain.

Methods

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (http://prisma-statement.org/) were followed [6]. We searched the PubMed database using the terms “Saharan dust” OR “desert dust” AND “health”. Studies selected for this review (after removing duplicates) were limited to those conducted on humans and written in English, and the final search took place on May 20th 2019. The search scheme is presented in **Figure 1**.

Results

Nineteen studies specifically investigated the health impacts of Saharan dust exposure on human health, as summarized in **Table 1**. Of these, seventeen examined populations in Southern Europe, specifically in the Mediterranean basin, which experiences proven increases in ambient PM levels recorded in air quality monitoring networks from Saharan dust due to proximity to the Sahara and atmospheric dynamics [7]. Two studies were studied Caribbean populations [8, 9]. All studies were epidemiological studies using a mixture of analytical techniques but mainly time-series analyses. Primary endpoints were mainly all-cause mortality or cause-specific (cardio-respiratory) mortality or, for those studies examining emergency admissions to hospital, either hospital admission rates [10-14] or asthma attacks [8, 9]. Since the effects of air-borne particles are related to their chemical composition and size, some studies investigated associations between health outcomes and different particle sizes but all studies included the coarse fraction (i.e., PM between 2.5 and 10 μm, $PM_{10–2.5}$).

The results can be summarized as follows. First, in general, PM levels were associated with an increased risk of all-cause and cause-specific mortality whether of desert or non-desert origin [11, 12, 15-19]. In those studies examining hospital admissions or disease-specific outcomes, a similar trend was seen, with increased PM concentrations associated with increased numbers of hospitalizations [10, 12] or hospital-treated asthma attacks [8, 9]. PM levels whether of desert or non-desert origin appear to have an impact on general and respiratory health.

Second, most but not all studies detected effects on outcomes attributable to the Saharan dust component of the detected PM. These effects tended to be stronger for cause-specific outcomes, i.e., those related to cardiovascular and respiratory morbidity. For example, Trianti et al. [13] observed that desert dust days were associated higher numbers of ER visits for asthma, chronic obstructive pulmonary disease and respiratory infections with increases of 38%, 57% and 60%, respectively ($p < 0.001$), while Staffoggia et al. [12] detected similar associations of mortality and hospitalizations with increases of desert and non-desert PM_{10} but stronger associations with desert dust for cardiovascular mortality (1.10%; 95% CI 0.16-2.06 compared with 0.49%; 95% CI –0.31-1.29 for non-desert dust). Similarly, Reyes et al. [11] reported that while periods without Saharan dust intrusions were marked by a statistically significant association between daily mean $PM_{2.5}$ concentrations and all- and circulatory-cause hospital admissions, periods with such intrusions saw a significant increase in respiratory-cause

admissions associated with fractions corresponding to PM_{10} and $PM_{10\text{-}2.5}$, while Alessandrini et al. [10] saw an effect modification of Saharan dust on the association between hospitalizations and particles for respiratory diseases and cerebrovascular diseases. Perez et al. [16], Mallone et al. [20], and Middleton et al. [19] in particular detected excess risk of cardiovascular events on Saharan dust days compared to non-dust days. In a study of children admitted with asthma in Guadeloupe, Caribbean [9], there were excess risk percentages for visits related to asthma on days with dust compared to days without dust [9.1% (95% CI 7.1–11.1%) versus 1.1% (95% CI 25.9–4.6%) for PM_{10} and 4.5% (95% CI 2.5–6.5%) versus 1.6% (95% CI 21.1–3.4%) for $PM_{2.5–10}$]. However, these results were not consistent, with Samoli et al. [18] detecting a negative between particle effects and mortality during dust events. Nevertheless, most of the available evidence appears to suggest that PM composed of Saharan dust contributes to excess adverse health outcomes.

The third major observation from the collated studies is that particle size has an impact on the observed health effects. Several studies observed significant impacts on all-cause or cause-specific mortality for PM_{10} (but not $PM_{2.5}$ or $PM_{10–2.5}$ [21]) and $PM_{10\text{-}2.5}$ (but not $PM_{2.5}$ [15, 22]). Coarser particle sizes appear to have a greater impact on mortality than smaller particle sizes.

However, not all studies reported the same pattern of findings. In individuals living in Gran Canaria Island, Spain, elevated Saharan dust levels did not exacerbate allergies in adult and elderly patients as assessed by the number of ER admissions, medication needs, and pulmonary function [14].

Conclusions

There are several theories on the mechanism underlying the impact of Saharan dust on human health. As a component of PM, dust particles may be inhaled. Accordingly, particle size has been proposed as a determinant of the potential of Saharan dust to cause health-related damage. Particles greater than 10 micrometers are generally not respirable, so the deleterious impacts of very coarse particles are likely to be external, for example by irritating the skin and eyes. Particles less than 10 micrometers (PM_{10}) in diameter, however, can be inhaled [4] and are therefore associated with respiratory disorders, given their direct contact with the upper respiratory tract. The smallest particles may enter the lower respiratory tract and eventually the

bloodstream, thereby exerting lower respiratory and cardiovascular effects. Details of the molecular and cellular events underlying the interaction of these particles with physiological systems are scarce. Non-desert dust particulate matter and other air pollutants are known to cause molecular and cellular alterations, for example, aberrant gene expression from exposure to particulate matter in general and anthropogenic pollutants specifically [23-26]. Such testing began at the bench using animal models and is now entering the clinical domain. Understanding how desert dust exposure interacts with specific tissues and cell populations at the molecular level could shed light on other health-damaging effects of dust exposure, deepen our knowledge of dust exposure beyond effects based on particle size, and provide opportunities for predictive tests or exposure biomarkers.

An interesting hypothesis is that infectious diseases disseminated through dust dispersion may also be responsible for adverse health effects. One way in which this might occur is that dust inhalation may damage protective mucosae, rendering individuals susceptible to bacterial infection [4]. Microbial populations and anthropogenic pollutants have been shown to travel on dust [3] and may contribute to outbreaks of infectious diseases such as meningitis [27], and some studies support this theory [28, 29]. One study compared the atmospheric microbiome on dust-affected and dust-free days by applying modern genomic techniques to investigate the impact of dust storms on the airborne microbial community [30]. Their results showed that the relative abundance of desert soil-associated bacteria increased during dust events, while the relative abundance of anthropogenic-influenced taxa decreased. Accordingly they concluded that dust storms enrich the ambient airborne microbiome with new soil-derived bacteria that disappear as the dust settles, suggesting that the bacteria are transported attached to the dust particles [30]. Similarly, recent investigations of desert dust composition suggest that toxic waste may be transported through the movement of desert dust [31-33]. Given that environmental regulations between countries from which the dust originates and the countries to which dust transports may significantly differ, this may present a fertile area of research with a significant impact on public policy and air quality standards [20].

An analysis of the microbial content of a Saharan dust event in Italy showed contamination of local soil with desert dust microorganisms, supporting the hypothesis that dust storms can move microbial communities from their origin to new environments [27]. Accordingly, two recent studies have linked infectious disease occurrence, specifically

meningitis, to Saharan dust movements. Diokhane et al. [29] conducted a study during winter and spring 2012 in Dakar, Senegal, which is part of the Sahelian zone, also referred to as the "meningitis belt". The number of meningitis cases was three times higher during this period compared to the same seasons in 2013. Notably, their investigation evidenced higher PM concentrations as well as elevated atmospheric dust loading during the period of increased meningitis cases. Perez Garcia-Pando et al. analyzed wind and dust information alongside seasonal incidences of meningitis in Niger and reported that these environmental conditions may predict meningitis outbreaks. In contrast, Woringer et al. [28] could not identify any association between epidemic meningitis in the "meningitis belt" and atmospheric dust load.

Finally, [35] recently reconstructed Saharan dust deposition over 240,000 years and in doing so demonstrated that present-day Saharan dust deposition is elevated compared to 5000 to 11,000 years ago. During that time, decreased dust in Saharan plumes may have contributed to the development of monsoon rains, and the effect of dust may continue to impact climatic change [35]. An in-depth understanding of Saharan dust deposition, its environmental impact, and its health-related sequelae may not only be relevant but increasingly urgent as both a public health and environmental concern.

In collating the available evidence in this mini-review, we have shown that (i) PM contributes to all-cause and cause-specific mortality and morbidity; (ii) PM arising from Saharan dust contributes to excess all-cause and cause-specific mortality and morbidity; and (iii) larger particle sizes may be more harmful than smaller particle sizes. Several associations exist between Saharan dust exposure and adverse health outcomes. As a PM component, Saharan dust is respirable and, as expected [2, 3], reportedly increases respiratory hospitalizations in patients with asthma, allergic disorders, and other respiratory diseases [8-11, 16, 36, 37]. The theorized association with cardiovascular illness [4] has been demonstrated in a subset of studies that show an increase in mortality due to cardiovascular causes upon dust exposure [20, 21, 37]. While the evidence supporting a health impact of Saharan dust exposure is emerging and fairly robust, the mechanisms underlying these associations remain elusive and require further study, perhaps by assessing blood-borne molecules, such as through gene expression or metabolite analyses, on Saharan dust days.

5 Πίνακας και Εικόνα της σχετικής δημοσίευσης:

Table 1. Published studies on the health effects of Saharan dust exposure.

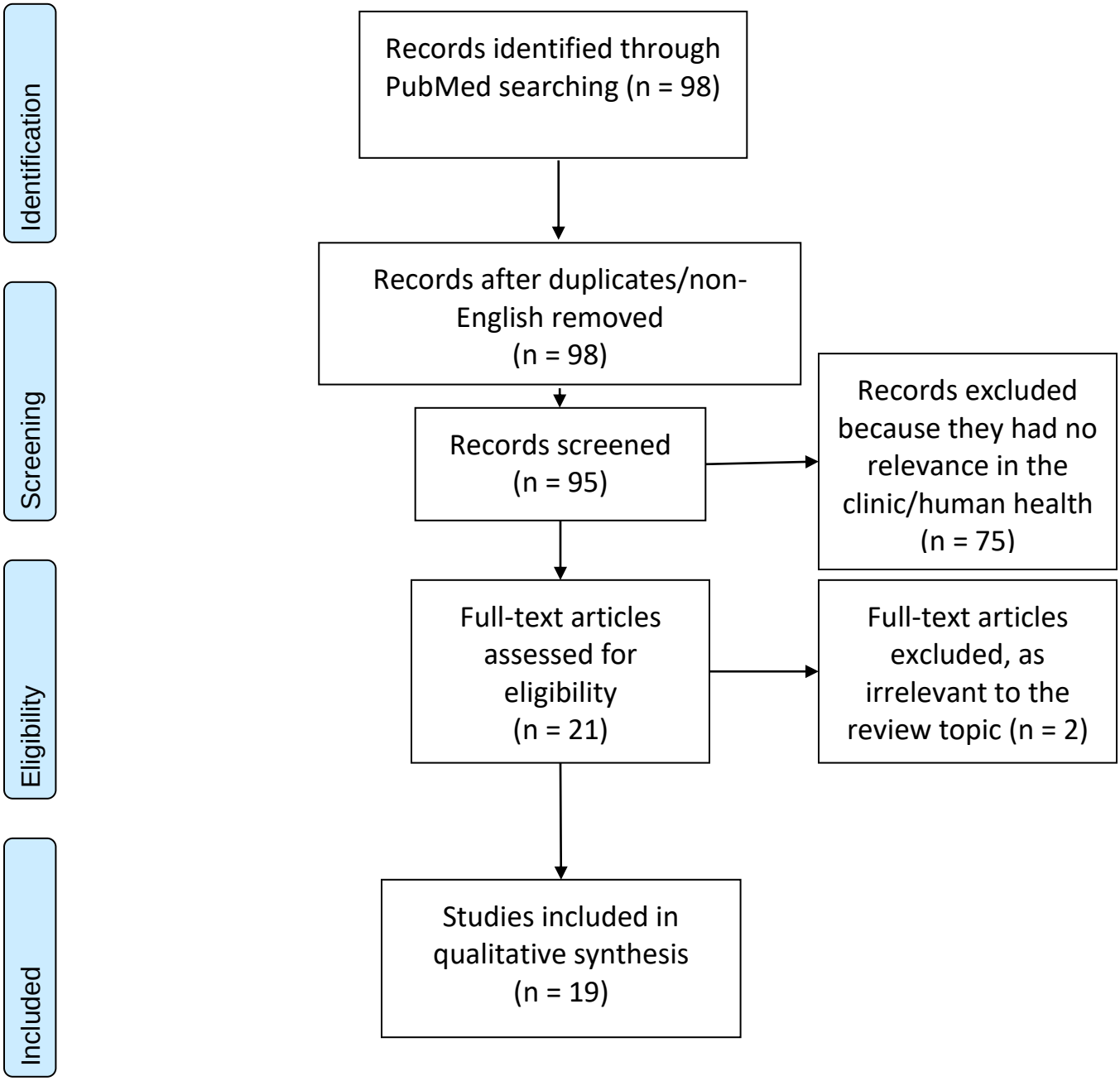
Reference	Location	Study design	Population	Health data source	Period of observation	Outcome	PM fraction	Results
Renzi et al. 2018 [17]	Sicily, Italy	Pooled time-series analysis	~5 million island inhabitants	Total island population using health regional databases	Jan 2006-Dec 2012	Cause-specific mortality	PM_{10}	Non-accidental mortality increased by 2.27% (95% CI = 1.41-3.14) and 3.78% (95% CI = 3.19–4.37) per 10 $\mu g/m^3$ increases in lag 0–5 non-desert and desert PM_{10} Significant associations with cardiovascular (2.4% (95% CI = 1.3-3.4) and 4.5% (95% CI = 3.8-5.3)) and respiratory mortality (8.1% (95% CI = 6.8-9.5) and 6.3% (95% CI = 5.4-7.2)
Trianti et al. [13]	Athens, Greece	Retrospective case-control	~4 million island inhabitants	Hospital databases	2001-2006	Daily ER visits, daily ER visits for respiratory diseases	PM_{10}	A 10 $\mu g/m^3$ increase in PM_{10} concentration was associated with 1.95% (95% CI 0.02-3.91%) increase in respiratory ER visits but not desert dust episodes Desert dust days were associated with higher numbers of ER visits for asthma, chronic obstructive pulmonary disease and respiratory infections with increases of 38%, 57% and 60%, respectively ($p < 0.001$)
Diaz et al. 2017 [38]	Spain, countrywide	Longitudinal time-series analysis	49 towns with >10,000 inhabitants	National mortality statistics	Jan 2004-Dec 2009	Mortality	PM_{10}	Particulate matter (PM) on days with intrusions associated with daily mortality in some region
Menendez et al. 2017 [14]	Gran Canaria, Spain	Prospective longitudinal; case-control	2854 adult emergency patients; 37 patients with allergies (asthma or allergic rhinitis)	In-hospital monitoring	Jan-Dec 2010	ER admissions, respiratory disease	$PM_{10\text{-}2.5}$, $PM_{2.5}$	No statistically significant relations were found between the allergic control group, the emergency room admissions, pulmonary conditions, medication, and elevated Saharan dust levels
Staffoggia et al. 2015 [12]	13 European cities in the Mediterranean basin	Retrospective cross-sectional	13 large European cities	Local mortality statistics and hospital discharge databases	2001-2010	Mortality and hospital admissions	PM_{10}, $PM_{2.5}$, $PM_{2.5\text{-}1.0}$	Associations of mortality and hospitalizations with 10 $\mu g/m^3$ increases of desert and non-desert PM_{10} were similar for all natural mortality (0.65%; 95% CI 0.24-1.06 and 0.55%; 95% CI 0.24-0.87), though association with desert dust stronger for cardiovascular mortality (1.10%; 95% CI 0.16-2.06 compared with 0.49%; 95% CI –0.31-1.29 for non-desert dust) and weaker for respiratory mortality (1.28%; 95% CI –0.42-3.01 compared with 2.46%; 95% CI 0.96-3.98)
Akpinar-elci et al. 2015 [8]	Grenada, Caribbean	Retrospective cross-sectional	4411 hospital visits, adults and children	Hospital records	Jan 2001-Dec 2005	Hospital-treated asthma attacks	NS	Variation in asthma was associated with change in dust concentration ($R^2 = 0.036$, $p < 0.001$)

Cadelis et al. 2014 [9]	Guadeloupe, Caribbean	Time stratified case-crossover	836 children 5-15 years	Tertiary hospital records	Jan 2011-Dec 2011	Hospital-treated asthma attacks in children	PM_{10}, $PM_{10-2.5}$	Excess risk percentages (IR%) for visits related to asthma in children aged between 5 and 15 years on days with dust compared to days without dust were, 9.1% (95% CI 7.1–11.1%) versus 1.1% (95% CI 25.9–4.6%) for PM_{10} and 4.5% (95% CI 2.5–6.5%) versus 1.6% (95% CI 21.1–3.4%) for $PM_{2.5–10}$
Reyes et al. 2014 [11]	Madrid, Spain	Ecological time-series	NS	Tertiary hospital records, ER admissions	Jan 2003-Dec 2005	All-cause and cardiovascular/respiratory emergency hospital admissions	PM_{10}, $PM_{10-2.5}$, $PM_{2.5}$	Periods without Saharan dust intrusions were marked by a statistically significant association between daily mean $PM_{2.5}$ concentrations and all- and circulatory-cause hospital admissions, periods with such intrusions saw a significant increase in respiratory-cause admissions associated with fractions corresponding to PM_{10} and $PM_{10-2.5}$
Alessandrini et al. 2013 [10]	Rome, Italy	Time-series analysis	NS; aged <14 years or >35 years	Daily hospital visits from regional public health database	2001-2004	All-cause and cardiovascular/respiratory emergency hospital admissions	PM_{10}, $PM_{10-2.5}$	Positive and statistically significant associations were found between $PM_{2.5–10}$ and cardiac diseases (for lag 0–1, 3.93%, 95% CI 1.58- 6.34) and between PM_{10} and cardiac, cerebrovascular, and respiratory diseases (for lag 0–1, 3.37%, 95% CI 1.11-5.68; for lag 0, 2.64%, 95% CI 0.06-5.29; for lag 0–5, 3.59%, 95% CI 0.18 -7.12). No significant effect was detected between $PM_{2.5}$ and either group of hospitalizations Effect modification of Saharan dust on the association between hospitalizations and particles was seen for respiratory diseases, with effects of $PM_{2.5–10}$ (14.62% vs −0.32, p=0.006). Effect modification of Saharan dust was also found for PM_{10} and cerebrovascular diseases (5.04% during dust-affected days vs 0.90% during dust-free days, p=0.143)
Neophytou et al. 2013 [39]	Cyprus	Time-series analysis	NS	National statistics	Jan 2004-Dec 2007	All-cause-mortality and cardiovascular/respiratory mortality	PM_{10}	A 2.43% (95% CI 0.53-4.37) increase in daily cardiovascular mortality associated with each 10 mg/m^3 increase in PM_{10} concentrations on dust days. Associations for total (0.13% increase, 95% CI: 1.03-1.30) and respiratory mortality (0.79% decrease, 95% CI: 4.69-3.28) on dust days and all PM_{10} and mortality associations on non-dust days were not significant
Perez et al. 2012 [16]	Barcelona, Spain	Time stratified case-crossover	~1.8 million	Local health registry	Mar 2003-Dec 2007	All-cause-mortality and cardiovascular/respiratory mortality	PM_{10}, $PM_{10-2.5}$, $PM_{2.5-1.0}$	During non-Saharan dust days, statistically significant effects of $PM_{10–2.5}$ for cardiovascular (OR=1.033, 95% CI 1.006–1.060) and respiratory mortality (OR=1.044, 95% CI 1.001–1.089) During Saharan dust days strongest cardiovascular effects were found for the same fraction (OR=1.085, 95% CI 1.017–1.158) with an indication of effect modification (p=0.111)
Diaz et al. 2012 [37]	Madrid, Spain	Time stratified case-crossover	NS	Local health registry	Jan 2003-Dec 2005	Daily cause-specific mortality	PM_{10}	The rise of mortality per 10 $\mu g/m^3$ PM_{10} concentration were always largely for Saharan dust days. No effects were found for cerebrovascular causes
Tobias et al. 2011 [22]	Madrid, Spain	Time stratified case-crossover	NS	Local mortality registry	Jan 2003-Dec 2005	Total mortality	$PM_{10–2.5}$, $PM_{2.5}$	During Saharan dust days, an increase of 10 mg/m^3 of $PM_{10–2.5}$ raised total mortality by 2.8% compared with 0.6% during non-dust days (p=0.0165). Effect not seen for $PM_{2.5}$

Mallone et al. 2011 [20]	Rome, Italy	Time stratified case-crossover	80,423 adults aged ≥35 years	Local mortality registry	Feb 2001-Dec 2004	All-cause-mortality and cardiovascular/respiratory mortality	PM_{10}, $PM_{10-2.5}$	Associations of $PM_{2.5–10}$ with cardiac mortality were stronger on Saharan dust days (9.73%; 95% CI 4.25–15.49%) than on dust-free days (0.86%; 95% CI –2.47-4.31%; p=0.005) Saharan dust days also modified associations between PM_{10} and cardiac mortality (9.55% increase; 95% CI 3.81–15.61%; vs. dust-free days: 2.09%; 95% CI –0.76-5.02%; p=0.02)
Samoli et al. 2011 [18]	Athens, Greece	Poisson regression	>4 million	National statistics	2001-2006	All-cause-mortality and cardiovascular/respiratory mortality	PM_{10}	A 10 μg/m^3 increase in PM_{10} was associated with a 0.71% (95% confidence interval (CI): 0.42% to 0.99%) increase in all deaths The main effect of desert dust days and its interaction with PM_{10} concentrations were significant in all cases except for respiratory mortality and cardiovascular mortality among those over 75 years. The negative interaction pointed towards lower particle effects on mortality during dust events.
Zauli Sajani et al. 2011 [40]	Emilia-Romagna, Italy	Time stratified case-crossover	~1.2 million	Regional mortality registry	Aug 2002-Dec 2006	All-cause-mortality and cardiovascular/respiratory mortality	PM_{10}	Respiratory mortality increased by 22.0% (95% CI 4.0-43.1%) on the Saharan dust day in the whole year model and by 33.9% (8.4- 65.4%) in the hot season model. Effects substantially attenuated for natural and cardiovascular mortality with ORs of 1.042 (95% CI 0.992-1.095) and 1.043 (95% CI 0.969-1.122), respectively
Jimenez et al. 2010 [21]	Madrid, Spain	Longitudinal, ecological, time-series	Subjects aged >75 years	Local mortality registry	Jan 2003-Dec 2005	All-cause-mortality and cardiovascular/respiratory mortality	PM_{10}, $PM_{10–2.5}$, $PM_{2.5}$	On Saharan dust days, a significant statistical association was detected between PM_{10} (though not $PM_{2.5}$ or $PM_{10–2.5}$) and mortality for all 3 causes analyzed, with RRs statistically similar to those reported for $PM_{2.5}$
Perez et al. 2008 [15]	Barcelona, Spain	Time stratified case-crossover	~1.8 million	Local mortality registry	Mar 2003-Dec 2004	Mortality	$PM_{10–2.5}$, $PM_{2.5}$	A daily increase of 10 μg/m^3 of $PM_{10-2.5}$ increased daily mortality by 8.4% (95% CI 1.5%-15.8%) compared with 1.4% (-0.8% to 3.4%) during non-Saharan dust days (p=0.05). In contrast, there was no increased risk of daily mortality for $PM_{2.5}$ during Saharan dust days
Middleton et al. 2008 [19]	Cyprus	Longitudinal, ecological, time-series	178,091	Inpatient admission data	Jan 2995 – Dec 2004	All-cause-mortality and cardiovascular/respiratory mortality	PM_{10}	For every 10 μg/m^3 increase in daily average PM_{10} concentrations, there was a 0.9% (95% CI 0.6-1.2%) increase in all-cause and 1.2% (95% CI -0.0-2.4%) increase in cardiovascular admissions All-cause and cardiovascular admissions were 4.8% (95% CI 0.7-9.0%) and 10.4% (95% CI -4.7-27.9%) higher on dust storm days, respectively

Abbreviations: CI=confidence interval; ER=emergency room; NS=not stated; OR=odds ratio; PM=particulate matter

Figure 1. PRISMA flow diagram for the literature search regarding the health effects of dust exposure.



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