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Historical background and Objectives

The *Saudi Journal of Health Research and Practice (SJHRP)* was established to address the growing demand for a platform dedicated to publishing high-quality, evidence-based health research in Saudi Arabia and beyond. As healthcare in the region undergoes rapid transformation in line with Vision 2030, the journal aims to bridge the gap between clinical practice and research. The goal is to empower healthcare professionals, educators, and researchers by providing a venue for sharing innovative findings and insights that address local and global health challenges.

By amplifying regional voices, the SJHRP aspires to contribute to the global health dialogue while ensuring that the unique perspectives and priorities of the region are represented.

Theme of the Issue

The second issue of the *Saudi Journal of Health Research and Practice* highlights the interplay between genetic, infectious, and psychosocial factors in health and disease. It features research on maternal separation stress and cognitive dysfunction, the prevalence of the XPG gene polymorphism among Saudi populations, a genetic evaluation of unilateral renal agenesis, and a rare case of primary thyroid tuberculosis. Together, these contributions reflect the journal's commitment to advancing interdisciplinary health research with a focus on regional and global relevance.

Editorial Message

It gives me great pleasure to introduce the second issue of the *Saudi Journal of Health Research and Practice*. Building on the momentum of our inaugural issue, this edition continues to reflect our mission of promoting high-quality, interdisciplinary research that addresses critical health challenges both locally and globally.

In this issue, we present a selection of original studies and case reports that explore the complex interplay between genetic, infectious, and psychosocial factors in health. The featured articles range from investigations into the cognitive effects of maternal separation stress, to genetic analyses of DNA repair mechanisms, to rare clinical case reports that broaden our understanding of disease presentation and management in the Saudi context.

Each contribution underscores the importance of integrating basic science with clinical application, and highlights the ongoing need for research that not only advances knowledge but also informs practice and improves patient outcomes. We are proud to see growing engagement from researchers in our region and beyond, and we remain committed to providing a platform that encourages rigorous inquiry, innovation, and collaboration.

I extend my deepest gratitude to our authors, reviewers, and editorial board members for their dedication and contributions, and to our readers for their continued support. We look forward to your engagement with this issue and to the ongoing journey of scientific discovery and advancement.

Professor Hussein M. Ageely
Editor-in-Chief
Saudi Journal of Health Research and Practice

About the Journal

The *Saudi Journal of Health Research and Practice (SJHRP)* is a peer-reviewed, open-access journal dedicated to publishing high-quality research that advances healthcare and evidence-based practice. The journal serves as a platform for researchers, clinicians, and academics to share knowledge and insights across a wide range of healthcare disciplines.

Journal Mission:

- To promote innovation and excellence in health research.
- To provide a platform for interdisciplinary collaboration.
- To address pressing health challenges both locally and globally.

Scope:

This journal covers all topics related to all aspects of health issues and healthcare research. Basic medical research with clear clinical implications will also be considered. Research fields of interest include but are not limited to:

- Public Health and Epidemiology
- Health Promotion and Disease Prevention
- Clinical Medicine Across All Specialties (including all clinical medical , dental and other clinical specialties and subspecialities)
- Pharmaceutical Research and Development
- Biomedical Sciences and Technology
- Mental Health and Behavioural Science
- Environmental and Occupational Health
- Quality of Care and Patient Safety
- Health Informatics and Digital Transformation
- Health Education and Behavioral Science
- Health Economics and Policy Research

Key Features:

- **Open Access:** Ensures free and unrestricted access to research for all readers.
- **Double-Anonymous Peer Review:** Guarantees a rigorous and unbiased review process.
- **Interdisciplinary Focus:** Publishes research from a variety of healthcare fields to encourage collaboration and innovation.

For more information, visit the journal's website at:
https://journals.jazanu.edu.sa/ojs/index.php/SJHR/index.

Announcements and Key Features

Announcements:

- SJHRP is currently accepting submissions for its next issue. Researchers are encouraged to submit their work via the journal's online portal.
- Plans for indexing SJHRP in major databases such as Scopus and PubMed are underway.

Key Features:

- **Open Access:** All published articles are freely accessible to readers worldwide.
- **Double-Anonymous Peer Review:** Ensures impartial and rigorous evaluation of submissions.
- **High Standards:** Committed to publishing impactful, evidence-based research.

Acknowledgments

The launch of the *Saudi Journal of Health Research and Practice (SJHRP)* is the result of the collaborative efforts and unwavering support of numerous individuals and institutions.

We extend our deepest gratitude to:

- **H.E. Professor Mohammad Aburassain**, President of Jazan University, for his visionary leadership.
- **Dr. Abdulkarim Meraya**, Vice President for Postgraduate and Research Affairs, for his guidance and dedication to advancing research at the university.
- **The Journal's Editorial Office**, for their tireless efforts in managing submissions and ensuring the journal's quality.
- **The Editorial Board Members**, whose expertise and commitment have been instrumental in shaping this journal.

Your contributions have made this milestone possible, and we look forward to your continued support as we advance together.

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Review Article

Pathophysiological Mechanisms of Maternal Separation Stress in Cognitive Dysfunction

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ABSTRACT Maternal separation (MS) animal stress models are commonly used to evaluate the factors affecting cognitive development. These models simulate mother–offspring separation during the lactation period because it is the most critical neurodevelopmental period for exploring the impact of early-life adversities on the risk of various neurological and cognitive disorders that can occur during adulthood. Cognitive dysfunction may result from multiple contradictory mechanisms that have not yet been reviewed. Therefore, in this narrative review, we aim to offer a critical and objective review of studies published in databases, such as PubMed, Google Scholar, and Web of Science, over the last 5 years that have used this stress model to propose a pathophysiological mechanism of MS-induced cognitive dysfunction. Our review reveals that MS induces cognitive dysfunction via the hypothalamic–pituitary–adrenal axis, which may alter neurogenesis and/or apoptosis. Furthermore, MS leads to neuroinflammation, oxidative stress, mitochondrial dysfunction, and accumulation of neuronal aggregates. In conclusion, the MS stress model offers a cost-effective method to evaluate potential therapeutic agents for a variety of neurodegenerative and neuropsychological disorders. Integrating multidisciplinary research into assessments is a comprehensive strategy for the early prevention of these disorders, which is one of the main objectives of the Saudi Arabia 2030 vision for efficient child integration in society.

Keywords: Hippocampus, Maternal Separation, Cognitive Dysfunction, Neuroinflammation, Oxidative Stress.

INTRODUCTION

Early-life adversities increase the risk of developing neurodegenerative, neuropsychological, depressive, and anxiety disorders [1]. Maternal presence during the early postnatal period is crucial for cognitive development, and depriving offspring of maternal care is a powerful early stress model commonly used to study factors affecting cognitive development [2]. This animal model of maternal separation (MS) induces stress by separating newborns from their mothers during the early postnatal period, with a time period ranging from 15 min to several hours, to induce neurological complications at the genetic and molecular levels [3]. MS during the early postnatal period can lead to long-lasting memory disorders, learning disabilities, and cognitive dysfunction due to hippocampus impairment [4]. Determining the effects of MS on cognitive dysfunction is challenging due to numerous variables, including stress patterns, age at stress induction, and stress duration and intensity [5]. Additionally, the combination of MS with other stress modalities obscures the pathophysiological mechanisms underlying the negative outcomes of MS. Despite the widespread use of animal models of MS in neurodevelopmental research, studies elucidating the pathophysiological mechanisms of MS-induced cognitive dysfunction are scarce. Furthermore, the differential outcomes of various factors, such as age at stress onset, sex-specific outcomes, and interactive neurological pathways, can lead to variable results. Therefore, through this narrative review, we aim to offer a comprehensive, critical, and objective review of the literature and identify studies that have used MS animal models to understand the pathophysiological mechanisms induced by MS that lead to cognitive dysfunction.

Inclusion/exclusion criteria: The databases of PubMed, Google Scholar, and Web of Science were searched for articles published in the last 5 years that used the MS animal stress model to propose a pathophysiological

mechanism of MS-induced cognitive dysfunction. We included papers that presented MS outcomes with respect to brain structures responsible for cognitive function, mainly the hippocampus, amygdala, prefrontal cortex (PFC), and dentate gyrus (DG). Studies that used the MS model to explain non-neurological outcomes were excluded. Older studies were included only if they showed distinctive results that no other studies had proposed regarding the pathophysiological mechanisms of MS. Therefore, this review is divided into sections to illustrate how MS affects cognitive and behavioral functions, specifically focusing on the hypothalamic–pituitary–adrenal (HPA) axis and important hippocampal physiological processes such as neurogenesis, programmed cell death, and apoptosis. It also includes sections explaining how MS might lead to neuronal inflammation, oxidative stress, mitochondrial dysfunction, and accumulation of harmful neuronal aggregates.

PATHOPHYSIOLOGICAL MECHANISM 1: MS AND HPA AXIS

Postnatal MS can affect neuroendocrine physiological responses to stress, potentially disrupting activities of the HPA axis [6]. Prevention of chronic glucocorticoid (GC) exposure protects the developing brain from the hyperactive HPA axis due to increased corticotropin-releasing hormone (CRH) signaling and disturbed negative hormonal feedback [7,8]. Gender differences can be observed in MS, altering glucocorticoid receptor (GR) and mineralocorticoid receptor expression, such as Npas4 and nuclear receptor subfamily 1 group D member (Nr1d1), in the hippocampus and PFC [9]. A previous study revealed that a periodic 3-h MS affected hippocampal CRH gene expression and baseline HPA axis activity at different time periods from the sixth postnatal day (PND), ultimately affecting cognitive function [8]. Even a single 24-h MS negatively impacts the behavior and cellular functioning of the bed nucleus of the stria terminalis CRH cells [10]. MS amplifies excitatory postsynaptic currents and reduces M-currents, which are crucial for maintaining membrane potential. It also increases the levels of CRH and pituitary adenylyl cyclase-activating cells, which are responsible for upstream CRH activity [11]. Extended MS significantly affects spatial working memory owing to increased corticosterone levels and neuronal loss in the DG [7]. Persistent memory impairment becomes evident during adulthood, even though GC, brain-derived neurotrophic factor (BDNF) receptor, or tropomyosin receptor kinase B levels are not impacted [12].

When combined with other stressors, MS may cause dysregulation of the HPA axis function and GR expression and lead to behavioral changes in both mothers and offspring. After weaning, MORC Family CW-Type Zinc Finger 1 (Morc1) expression decreased 4 h after MS in mothers, whereas the expression of GR, nuclear receptor subfamily 3 (Nr3c1), remained unaffected. Levels of serum gamma-aminobutyric acid (GABA), but not glutamate, were significantly increased, supporting subclinical indicators of postpartum depression [13]. HPA axis dysfunction can also be attributed to the restriction of pub-mother nutritional needs, which impacts cognitive well-being and causes oxidative stress through hyperactivity of the HPA axis [14]. A 3-h MS combined with restricted bedding leads to anxiety-like behaviors and increased hypothalamic CRH levels [15]. Six hours of MS with limited bedding leads to an upregulation of the CRH gene, with modification of stress response genes, resulting in increased expression of CRH receptor 1, CRH receptor 2, and serotonin receptor 5-hydroxytryptamine-1A, and decreased expression of Nr3c1 and Htr1a in the hippocampus [16]. In contrast, comparing a single 24-h MS trial with a chronic 3-h MS trial showed no effects on the expression of stress-related CRH, CRH receptor 1, CRH receptor 2, Nr3c1, Nr3c2, and serum corticosterone at the end of the second postnatal week in the DG [17]. Twenty-four-hour MS lowered the expression of catechol-o-methyl transferase, which is involved in regulating GCs. Increased numbers of glutamic acid decarboxylase 67-positive neurons were found in the DG, hippocampus cornu ammonis (CA3), CA1, subiculum, presubiculum, and parasubiculum. Further, there were also more parvalbumin-positive cells in all areas except the CA1 [3]. Notably, a single MS trial on the third PND elevated BDNF expression in CRH neurons, suggesting a heightened level of neural plasticity [18]. MS reduces responses in the novel object recognition task by lowering the expression of Nr1d1 in the dorsal hippocampus [19].

MS can also affect adult learning and memory by disrupting developmental shifts in GR expression, which typically decreases during the second and third postnatal weeks, but 1 h of MS slows down this reduction and increases GR expression during weaning [20].

GR is found in the cytoplasm with a group of proteins, including FK506-binding proteins 51 and 52, which promote affinity and facilitate action via microtubule-protein interactions. However, 4 h of MS treatment increases mRNA expression in the amygdala and hippocampus [21]. Moreover, mice exposed to MS show increased repressive methylation of the GR exon 17 promoter and decreased histone acetylation, indicating long-lasting negative effects on behavior due to postnatal MS histone modification [22]. c57BL6 mice exposed to MS and social defeat stress showed that both stressors significantly influenced genes involved in histone methylation, opioids, neurotrophins, and GC signaling pathways, suggesting potential molecular mechanisms for cognitive and behavioral regulation [23]. Quantitative polymerase chain reaction results revealed that 3 h of MS caused variations in the expression of transcripts associated with GC signaling, a significant reduction in the Fkbp5 transcript, and an elevated Ptges3 transcript [18]. Females showed more Fos-labelled neurons than males; however, these findings cannot be solely attributed to MS, as they were observed under combined stressors [24].

Generally, combining MS with other stress methods may be useful to mimic specific disease conditions; however, this makes it more difficult to understand the specific adversities of the MS stress model itself.

Now that we have discussed the effects of HPA axis dysfunction caused by MS, we can examine how it affects neurogenesis, since it has been suggested that this dysfunction can directly disrupt normal neurogenesis.

PATHOPHYSIOLOGICAL MECHANISM 2: MS AND NEUROGENESIS AND APOPTOSIS

Neurogenesis is a neuronal physiological process that involves different stages of formation of new neuronal cells and is believed to occur throughout life in the DG of the hippocampus. The postnatal development of the hippocampus involves extensive neurogenesis, synaptogenesis, myelination, and microglial maturation [25]. Increased vulnerability to cognitive impairment in adulthood may result from aberrant adult neurogenesis, hypomyelination, or oligodendrocyte dysfunction [26]. An increase in CRH during critical periods causes poor dendritic branching, reduced myelination, and neuronal conductance owing to the HPA axis feedback deficit [10]. Corticosterone cytotoxicity induced by 15-min MS increases the risk of hippocampal abnormalities due to decreased neurogenesis or increased neuronal apoptosis in the CA3 region of the hippocampus [9]. Corticosterone cytotoxicity mediates apoptosis by activating protein kinase A, protein kinase C, calcium/calmodulin-dependent kinase II, and extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) [27]. In addition, postnatal MS inhibits neural stem cell proliferation, impairs survival, and alters newborn cell fate in the DG [60]. Even short-term MS can significantly reduce synaptosomes and increase the number of immature spines in the CA1 region of 1-month-old mice. Moreover, it affects synaptic maturation in juvenile mice by decreasing protein production for axonal growth and myelination [28]. Daily 3 h of MS impacts hippocampal granule cell neurogenesis; however, its impact on the neurogenic niche remains uncertain [56]. Based on a previous study that showed reduced hippocampal volume due to impaired synaptic plasticity, 6 h of MS led to enlarged gray matter volume in the amygdala on magnetic resonance imaging in late adulthood, but not in early adulthood or immediately following MS [29]. Three hours of MS affected locomotor activity, spatial memory, and recognition memory due to a reduced neuronal count in the hippocampus, as evaluated by the mean number of cells expressing doublecortin and Ki67 [30]. MS reduced dorsal hippocampal neurogenesis only in young adults, while 10-month-old mice exhibited a similar decline in dorsal and ventral hippocampal neurogenesis. These findings were attributed to MS-induced insulin sensitivity [31]. Further, expression of the excitatory neurotransmitter N-methyl-D-aspartate receptor subunit GluN1 decreased with an increase in the quantity and diameter of dark neurons in CA3, suggesting MS-induced neuronal loss [32].

At the end of the second postnatal week, MS-induced changes in gene expression are associated with myelin-ensheathment genes in the medial PFC [33]. In addition, western blot analysis has revealed that adult anxiety-like behavior is related to persistent atypical DNA methyltransferase changes in the hippocampus of MS-exposed mice [34]. Further research is required to determine whether brief MS indirectly inhibits myelination or spontaneous firing activity [35]. The glial cell phenotype analysis showed that MS increased DG proliferation in females to a greater extent than that in males [36]. Asymmetrical synapses with a thicker postsynaptic density than that of the presynaptic fraction facilitate hippocampal plasticity. In rat models of depression exposed to MS for 3 h, presynaptic density and cleft length were reduced [37].

In contrast, MS improved learning and memory and increased dendrite length, terminal tips, and spine density, particularly in thin spines, as evidenced by electroporation, three-dimensional reconstruction, immunohistochemistry, and BrdU labeling [38]. Additionally, MS increased the number of undeveloped DCL neurons in mice lacking the methyl-CpG-binding protein gene, which regulates gene expression and neuronal plasticity [39]. Interestingly, this effect was not evident in females heterozygous for methyl-CpG-binding protein, who showed less anxiety-like behaviors [40]. This study revealed a reduction in reelin levels in mutant rats for the first time [39]. The opposing effects of MS on the hippocampus could be influenced by the age of onset as well as by various brain regions and the variability of staining methods.

MS can affect neurogenesis through Ca^{2+}-binding proteins. For instance, MS reduces the density of interneurons expressing the Ca^{2+}-binding proteins parvalbumin, calbindin, and calretinin in the amygdala and nucleus accumbens [41]. A study found that 3 h of MS reduced exploratory behaviors among males and caused spatial memory loss in both males and females; however, calretinin and calbindin-D28k levels differed between sexes [42]. MS led to increased arginine vasopressin expression and decreased levels of GABAergic interneuron markers, including parvalbumin, and calbindin-D28k. A reduction in cortical Ca^{2+}-binding protein-28k-positive cells and an increase in cortical ionized Ca^{2+}-binding adaptor-positive cells was observed, confirming the early-life stress paradigm [6]. A study involving MS and calbindin-D28K in the hippocampus found a significant increase in these substances and a reduction in the medial basal hypothalamus, indicating a dysfunctional negative feedback of the HPA axis [43]. Although this study did not meet the inclusion criteria in terms of publication year, it is the only study to demonstrate the effect of MS on calbindin-D28K in the hippocampus. Besides, 24-h MS reduced the number of parvalbumin-expressing interneurons in the CA1 and PFC

and reelin-expressing interneurons in the CA1 and CA3 regions of the hippocampus. However, cellular apoptosis did not show any effect, suggesting the downregulation of markers rather than the depletion of interneurons. Immunohistochemistry targeting glutamatergic and inhibitory vesicular transporters showed reduced inhibitory synapses in the CA1 and CA3 areas; however, excitatory synapses remained unaltered. Therefore, modifications to the inhibitory circuitry caused by MS appear to be cellular and region-specific [44].

MS for 1 h postnatally reduced microglial ramifications, led to poor phagocytosis of synaptic debris, and decreased the expression of receptors known as triggering receptors expressed on myeloid cells-2. Elevated phagocytic activity has been linked to increased spine density in the CA1 pyramidal neurons, suggesting that adequate hippocampal stimulation between the second and third weeks of infancy is crucial for microglial ramification and synaptic pruning, which can affect hippocampal function and neuronal connectivity [45]. The physiological transformation of ameboid microglia into ramified microglia is impaired by early-life stress in a region-dependent manner. Antibodies against NeuN, microglia, Ki67, and doublecortin have shown that the MS group has more Ki67-positive cells in the DG and larger densities of ameboid and intermediate microglia in the PFC [17].

Normal hippocampal development relies on apoptosis or programmed cell death, which can be affected by the adverse effects of MS [46]. MS may lead to medial PFC apoptosis; however, the mechanisms underlying this effect are unclear [47]. Growing evidence suggests that the autophagy–lysosomal pathway can lead to increased protein deposition and neurodegeneration [48]. MS alters the autophagy–lysosomal pathway, potentially dysregulating proteostasis and affecting the onset and progression of neurodegenerative diseases [48]. Three hours after MS, learning and memory retrieval are impaired by increased apoptotic cell death and decreased early-phase protein kinase B (AKT) phosphorylation in the DG region. During adulthood, MS rats show lower GR1 expression in the DG, suggesting that behavioral alterations may link MS-induced pathological alterations to the inhibition of the AKT pathway and neuronal apoptotic death [49]. Analysis of hippocampal neuronal death in MS-induced depressive behavioral changes showed increased mRNA levels of Bax, caspase-3, and caspase-9 and decreased expression of BCL-2 and BDNF. However, these changes were reversed by treatment with a mitogen-activated protein kinase inhibitor. This study suggests that the death of hippocampal neurons may be mediated by the ERK1/2 signaling pathway, potentially opening new treatment options for neurodegenerative disorders [50]. The Majcher–Maślanka study revealed that MS rats had more numbers of astrocytes and NG2 glial cells but fewer microglial cells. Proapoptotic gene expression was lowered, whereas pro-survival gene expression was increased, suggesting potential interference with neuronal apoptosis [47].

Having discussed the effects of MS on neurogenesis, we now review MS-induced neuronal inflammation.

PATHOPHYSIOLOGICAL MECHANISM 3: MS AND NEUROINFLAMMATION

MS is a reliable model for studying the pathophysiology of mental and behavioral disorders influenced by neuroinflammation. Three hours of MS increased microglial activation in a traumatic brain injury model, indicating increased neuroinflammation but reduced cell proliferation in the ipsilateral neurogenic niche, and impacted the survival of cells in the hippocampus on the same side of the injury [51]. Cognitive behavior was not obviously changed due to MS, but depression-like behaviors increased when they were exposed to secondary adulthood stress. MS increases pro-inflammatory markers, decreases anti-inflammatory parameters, activates microglia, and facilitates pro-inflammatory transitions in the hippocampus. Moreover, changes in neurogenesis and BDNF expression in the hippocampus have been linked to inflammatory processes [52].

Limited research has been performed to understand how early MS and sex affect blood–brain barrier (BBB) function and immune system response to lipopolysaccharide-induced neural inflammation [46,47]. Nicolas et al. studied the effects of MS and lipopolysaccharides on hippocampal neurogenesis and inflammatory responses in juvenile female rats. They discovered that MS increased the levels of the pro-inflammatory cytokine interleukin (IL)-1 in the ventral hippocampus. In contrast, MS lowered microglial activity in both the dorsal and ventral hippocampi. Neither MS nor lipopolysaccharide had an effect on new neuron generation or distal dendrite density [53]. Increased BBB permeability leads to proinflammatory responses in the hippocampus. Separated male pups showed an elevated neuroinflammatory response, whereas separated female pups showed no difference, further suggesting that MS accelerates neurodegenerative onset through neuroinflammation in a sex-specific manner [47]. MS attenuates the activity of prostaglandin-producing enzymes, COX-2, prostaglandin endoperoxide synthase, and chemokines (CXCL-1 and MCP-1), without elevating plasma cortisol levels, leading to increased susceptibility to depressive-like behavior [54]. MS causes depressive-like upregulation of inflammatory genes IL-1β and TNF-α in the hippocampus [55]. Furthermore, MS causes sex-specific variations in chemokine motif ligand 1 and TNF levels as early as the second postnatal week [56]. MS activates microglia in the hippocampus, amygdala, and PFC due to high levels of inflammatory cytokines, especially IL-17 [57]. Changes in the levels of pro-inflammatory cytokines were found to depend on sex and age. A previous study showed that IL-1β and IL-10 levels decreased in 1-month-old animals, whereas IL-4 elevation

was observed around 3 weeks postnatally. Thus, circulating cytokine and parvalbumin levels could be used as early signs of cognitive decline in adulthood [58]. However, studies on the effects of MS on these cytokines in MS-induced behavioral deficits are scarce.

MS-induced neuroinflammation affects mitochondrial function, which protects neurons from oxidative stress toxicity. The next section reviews the mechanism by which MS induces mitochondrial dysfunction.

PATHOPHYSIOLOGICAL MECHANISM 4: MS AND MITOCHONDRIAL DYSFUNCTION

Over 100 stress-related proteins were upregulated, whereas almost 140 were downregulated, revealing an enrichment of proteins linked to mitochondrial dysfunction in a MS animal model combined with social isolation or chronic restraint stress [59]. MS models exhibited damage to mitochondrial membrane proteins and lipids, disruption of electron transport chain enzymes, and impaired expression of mitochondrial genes [60]. Fission and fusion are crucial functions of mitochondria. Function is controlled by the fission proteins dynamin-1-like protein (Drp1) and fission-1 (Fis1), whereas fusion is controlled by mitfusin1 (Mfn), Mfn2, and optic atrophy-1 (OPA1) proteins [61]. A 3-h MS model with reduced synaptogenesis and neuronal synapse formation was associated with mitochondrial damage and lowered ATP levels in the hippocampus. MS also affected Mfn2, Drp1, and fission-1 expression. Western blotting revealed reduced Mfn2 expression, whereas the levels of the fission mediators Drp1 and fission-1 were elevated [37].

MS can increase reactive oxygen species levels and mitochondrial respiratory chain complexes I, II, III, and IV and induce depressive behavior with upregulation of glutamate dehydrogenase-1, which is the determinant of mitochondrial energy production, and downregulation of the isocitrate dehydrogenase [NAD] alpha subunit [60]. These enzymatic changes cannot be attributed solely to the MS effect, as they were observed in a chronic mild stress model. A deeper understanding of the MS modification of certain brain parameters is necessary to identify phenotypes and biomarkers linked to mitochondrial dysfunction as prophylactic, diagnostic, and novel therapeutic approaches for cognitive dysfunction.

PATHOPHYSIOLOGICAL MECHANISM 5: MS AND OXIDATIVE STRESS

Long-term exposure to MS can alter the HPA axis and mediate neuroinflammation and oxidative stress, leading to the emergence of neurodegenerative diseases [62]. Three hours of MS in the first 2 postnatal weeks induced depression and anxiety-like behaviors, which were linked to enhanced lipid peroxidation by elevated malondialdehyde (MDA) and nitric oxide levels, as well as a reduction in antioxidant capacity in the brain [14]. Three hours of MS followed by multifactorial stress in adulthood resulted in disturbed cognitive test results, with reduced levels of antioxidative enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPX) and increased MDA levels [63]. Soares et al. employed 4 h of MS in the preweaning period and examined parts of the brain for antibodies against parvalbumin and 8-oxo-dG, which are markers of oxidative DNA damage. They found that 8-oxo-dG and parvalbumin levels were higher in the PFC, basolateral amygdala, and hippocampus in a sex-dependent manner [14]. MS affects the oxidative markers and behavior of adult mice, showing anxiety-like behaviors, as well as long-term lowering of GPX, catalase, and thiobarbituric acid reactive substances [62]. Additionally, low levels of TrkB, nitrite, BDNF, and antioxidant activity have been observed in the hippocampus 3 h after MS [64]. MS extended for 6 h leads to high serum corticosterone and MDA levels and reduced total SOD and GPX levels [65]. The interpretation of these changes cannot be limited to MS alone, because several stress variables were employed in the experimental design. After reviewing the effects of MS on oxidative stress, we explored its effects on the accumulation of toxic aggregates in neurons.

PATHOPHYSIOLOGICAL MECHANISM 6: MS AND ACCUMULATION OF NEURONAL AGGREGATES

Postnatal stress increases the production of amyloid precursor protein (APP) and amyloid-beta (Aβ) peptides, which can change cognitive function. Three hours of MS in APP-wild-type male transgenic mice led to cognitive deficits, as observed in the Morris water maze test, due to higher senile plaques and lower cholinergic neurons in the hippocampus. APP heterozygous mutant mice exhibited significant microglial activation, early Aβ plaque formation, increased vessel-associated microglia, and disruption of the BBB [66]. It has been revealed that Aβ peptides can directly stimulate CRF neurons, increase levels of Aβ 40 and Aβ42, increase expression of beta-site amyloid precursor protein cleaving enzyme 1 (BACE1), and phosphorylate Tau in the hippocampus potentially causing accumulation of neuronal aggregates. The combination of MS-induced microglial activation and APP mutation may impede the vascular inflammatory system and accelerate the progression of cognitive dysfunction in a sex-specific manner. MS female mice models showed increased premature Aβ peptides accompanied by changes in BDNF, cytoskeleton-associated protein expression, and microglial activation in the PFC [56].

The following diagram summarizes the main pathophysiological mechanisms of MS.

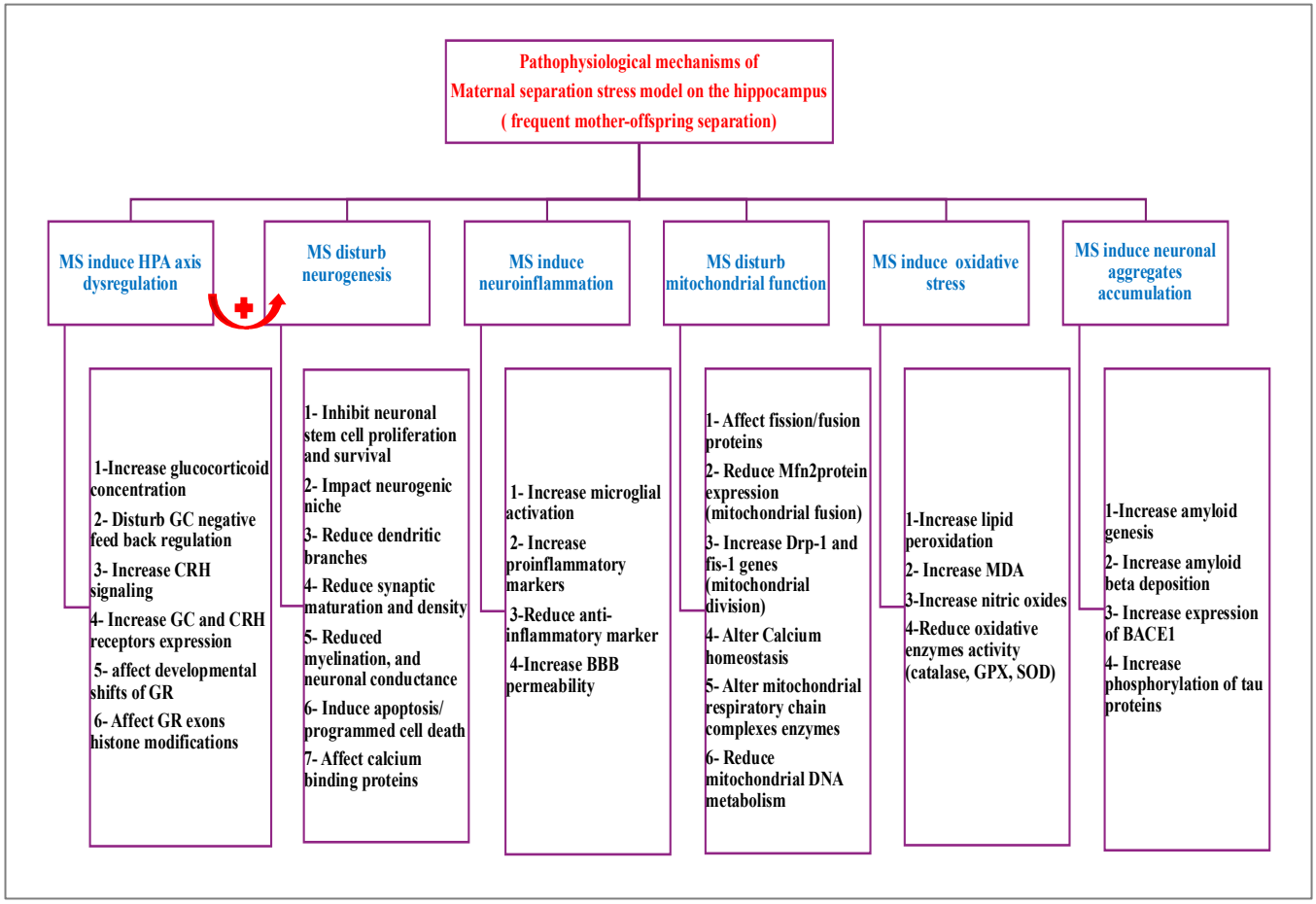

Figure 1: Pathophysiological mechanisms of MS-induced cognitive dysfunctions**.** The figure illustrates the different mechanisms and pathophysiological outcomes of maternal separation. + refers to induce. *MS:* maternal separation *HPA:* hypothalamic- pituitary- adrenal axis *GC:* glucocorticoid *CRH:* corticotropin-releasing hormone *GR:* glucocorticoid receptors *BBB:* blood brain barrier *Mfn2:* mitofusin 2 *Drp-1:* dynamin related protein *MDA:* malonaldehyde *GPX:* glutathione peroxidase enzyme *SOD:* superoxide dismutase *BACE1:* β-site amyloid precursor protein cleaving enzyme.

CONCLUSION

Frequent mother–infant separation during early infancy can lead to neurological alterations and various disorders. MS during the early postnatal period can cause memory disorders, learning disabilities, and cognitive dysfunction owing to impaired hippocampal function. Factors such as sex, age, and MS protocol affect the sensitivity of the hippocampus to stress hormones. Combining MS with other stressors can increase vulnerability to cognitive dysfunction in adulthood owing to hippocampal neurogenesis, inflammatory processes, oxidative disorders, and induced apoptosis. Furthermore, the differential outcomes of various factors, such as age at stress onset, sex-specific outcomes, and interactive neurological pathways, can lead to variable results.

The MS stress model is a cost-effective and simple tool that supports the understanding of cognitive disorders, particularly those caused by early childhood stress. It can induce various cognitive, memory, and neuropsychological disorders, such as Alzheimer's disease, Parkinson's disease, dementia, anxiety, and depression. The model analyzes protein, genetic, and molecular factors to understand the pathological outcomes of early-life adversities. Future studies should use the MS stress model to understand the alterations caused in certain brain parameters to identify phenotypes and biomarkers. Utilizing the MS stress model in conjunction with prophylactic, diagnostic, and therapeutic approaches can lead to the discovery of potential therapeutic agents for a variety of neurodegenerative and neuropsychological disorders.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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Original Article

Prevalence of NER DNA repair gene XPG rs17655 C>G polymorphism among Saudi Populations: A Comparative Study with Global population

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ABSTRACT Environmental toxins damage DNA, increasing the risk of cancer if not repaired. Xeroderma pigmentosum group G (XPG) is essential for nucleotide excision repair (NER). The XPG exon 15 C>G polymorphism may influence this process, increasing the risk of cancer. We examined the frequency of the XPG exon 15 C>G polymorphism in the Saudi population and compared it with that in other populations. By conducting a PUBMED search, we identified epidemiological studies across different ethnic groups. Allele and genotype frequencies were determined, and statistical analysis was performed using the SPSS 21 software. In Saudi Arabia, the frequency of the mutant allele G was 57%, which was higher than that reported in the USA, Brazil, and China. However, higher frequencies of G alleles were found in Germany (79%), India (65%), Italy (75%), Romania (78%), Spain (75%), and Tunisia (61%). The frequency of the GG genotype in Saudi Arabia was 32%, which was higher than that in the USA (5%), Brazil (12%), and other countries but lower than that in Germany, India, Italy, Romania, Spain, and Tunisia. It was found that the XPG exon 15 C>G polymorphism has a unique frequency pattern in Saudi Arabia, probably due to ethnic differences. This study provided insights into the role of GG variants in the progression and therapeutic response of cancer, leading to improved treatments for the Saudi population.

Keywords: NER DNA Repair Genes, XPG, Polymorphism, Genotype, Saudi Population.

INTRODUCTION

Globally, cancer rates and fatalities are increasing rapidly, making cancer a public health concern (1). The incidence of cancer in the Saudi population is greater among those who lead sedentary lifestyles, consume processed meals, consume high-calorie foods, and smoke (2). Several factors are strongly associated with the development of cancer, including smoking, alcohol consumption, irregular lifestyles, genetics, and environmental factors; however, environmental carcinogens cause DNA damage, which may subsequently cause genomic instability and result in the development of cancer, and the molecular mechanisms underlying the genesis of cancer remain poorly understood. The DNA repair pathway may be affected by specific DNA repair gene polymorphisms, either by themselves or along with environmental factors, increasing an individual's chance of developing cancer. Knowing the genetic frequency of DNA repair gene polymorphisms in the population that affect cancer susceptibility may help researchers identify cancer, develop new therapeutic approaches, and predict the prognosis of this fatal illness.

The genetic DNA of organisms is continuously harmed by various external factors and byproducts of cellular metabolic processes, such as reactive oxygen species. DNA damage can result in mutations, genomic instability, and a greater risk of cancer if it is not repaired. Cells have developed intricate DNA repair processes to protect genome integrity and guarantee appropriate cellular function in response to these assaults (4). Deficiencies in genetic repair capabilities can affect how the body responds to chemicals that harm DNA and aid in the onset or spread of cancer. Genes associated with DNA repair pathways are thought to be candidate genes for cancer susceptibility. "Polymorphisms in DNA repair genes may reduce the DNA repair capacity (DRC) of certain individuals compared to that of the general population." (5, 6). This emphasizes the importance of the genetic variables of hosts as determinants of individual DNA repair capability, which increases the susceptibility of the population to malignancy.

"Nucleotide excision repair (NER), an important DNA repair pathway, is an essential and adaptable system that tracks and fixes a range of DNA damage, such as bulky adducts, UV-induced cyclobutane pyrimidine dimers, and DNA cross-links" (7). Mutations in NER pathway genes may trigger changes in the risk of cancer. XPG is found on chromosome 13q22-q33 and encodes a structure-specific endonuclease with a length of 1,186 amino acids (8). This protein is essential for identifying DNA damage and attaches to and cleaves damaged DNA very early on to accelerate the process of DNA repair later. "Additionally, XPG is implicated in RNA transcription through interaction with other transcription activator complexes, which eventually influence mutagenesis and cell death" (9, 10). Single nucleotide polymorphisms (SNPs) in the XPG gene have been linked to an increased risk of developing different types of cancer in different populations.

A faulty XPG causes DNA repair issues, which in turn cause genomic instability, gene dysfunction, and the start of carcinogenesis (11). *XPG* is highly polymorphic. Among known polymorphisms, a nonsynonymous Asp1104His (C>G) polymorphism (rs17655) at codon 1104 in exon 15 may affect protein activity and interaction with TFIIH and XPG function, thereby affecting NER function and DRC and altering genetic integrity and susceptibility to cancer (12). Therefore, the onset and progression of cancer may be intimately associated with genetic changes in NER-related genes.

Interest in the molecular genetics of cancer in Saudi Arabia has increased in recent years, and several studies have focused on DNA repair genes. These findings suggest that the *XPG* gene may be a useful predictive molecular genetics biomarker for cancer. This study determined the prevalence of the *XPG* exon 15 C>G polymorphism in a normal healthy Saudi population and compared it to that reported in a sufficient number of epidemiological studies in other populations worldwide. This is the first study to compare the frequency of XPG exon 15 C>G DNA repair gene polymorphisms among the Saudi population and other populations worldwide.

MATERIALS AND METHODS

Subjects

Prevalence of gene variants

We searched MEDLINE and PUBMED by applying "*Xeroderma pigmentosum group G*", *"Excision repair cross-complementation group* 5", "XPG", "*XRCC5*", "polymorphism", "genetic variant", "cancer", and "carcinogenesis". There were no language restrictions, and the search was restricted to human topics. Only the genotype frequencies of the control population were considered for case-control investigations. We excluded articles that did not disclose genotype frequencies, only allele frequencies were included.

Statistical analysis

"Pearson's $\chi 2$ test was performed to compare the genotype and allelic frequencies of different populations using the SPSS software (version 21). Court Lab (web-based software) was used to examine the Hardy-Weinberg equilibrium. All results were considered to be statistically significant at $P < 0.05$.

RESULTS

We identified 15 publications (13–27) reporting the frequency distribution of the XPG exon 15 G>C polymorphism in different populations, which were subsequently compared to the Saudi population. The frequency distributions of three genotypes and alleles of the *XPG* exon 15 G>C gene polymorphism in different populations with reference to Saudi Arabia were compared via the $\chi 2$ test (Table 1). The variant genotype GG frequency in the Saudi population was 32%, which was higher than that in the USA (5%), Brazil (12%), China (20.4%), Czech Republic (4.3%), France (2%), Korea (28.6%), Poland (5%), and Turkey (9.4%). The frequency of the variant genotype GG was greater in Germany (62%), India (42%), Italy (56%), Romania (61%), Spain (55%), and Tunisia (36%) than in the Saudi Arabian population. A significant distribution of the variant genotype was noted in the USA, Brazil, Czech Republic, France, Germany, Italy, Romania, Spain, and Turkey compared to the Saudi Arabian population. However, no significant differences were found in China, India, Korea, or Tunisia. The frequency of the variant allele G in the Saudi Arabian population was 57%, which was higher than that reported in the USA, Brazil, China, the Czech Republic, France, Poland, and Turkey. However, the frequency of variant allele G was greater in Germany (79%), India (65%), Italy (75%), Romania (78%), Spain (75%), and Tunisia (61%) than in the Saudi Arabian population.

DISCUSSION

"Single-nucleotide polymorphisms (SNPs) are the most common form of variation in the human genome, which can alter the level of expression or function of genes or their encoded products and thus determine the phenotype of the organism" (28). "SNPs in DNA repair genes can influence the level of DNA damage, individual DNA repair capacity (DRC), and cancer risk" (29). Consequently, SNPs are necessary for the mechanisms underlying cancer (30). "Many studies have shown that exposure to exogenous and endogenous carcinogens (DNA-damaging chemicals) as well as the genetic profile or "genetic makeup" of individuals determine their risk of developing cancer. This genetic susceptibility may result from inherited polymorphisms in genes involved in carcinogen metabolism and repair of DNA damage" (31).

"Damage identification, damage demarcation and unwinding, damage incision, and new strand ligation are the phases of NER. More than 30 components are involved in this intricate process, and each step requires corresponding

functioning proteins" (32). "Polymorphisms of NER genes can further alter the NER process by influencing the expression and function of key proteins in the NER pathway. Thus, polymorphisms in the NER genes might be associated with genetic susceptibility, chemotherapeutic sensitivity, and the prognosis of cancer (33). Information from healthy individuals is needed to assess the significance of these genetic markers in the vulnerability, expression, prognosis, and treatment of illness because DNA repair gene polymorphisms are distributed significantly differently across different ethnic groups. Since ethnicity affects a person's susceptibility to specific diseases, it is necessary to investigate how NER gene genotypes and minor alleles vary across different groups because these genes are essential for maintaining the accuracy of the genome.

In this study, the Saudi population had a 32% variant genotype GG frequency, which was higher than that found in the USA, Brazil, China, Czech Republic, France, Korea, Poland, and Turkey. However, compared to the Saudi Arabian population, individuals in Germany, India, Italy, Romania, Spain, and Tunisia had higher frequencies of the variant genotype GG. A significant frequency distribution of variant genotypes was found when the Saudi Arabian population was compared to the USA, Brazil, the Czech Republic, France, Germany, Italy, Romania, Spain, and Turkey. However, no significant findings were recorded in China, India, Korea, or Tunisia.

The Saudi Arabian population has a 57% frequency of the mutant allele G, which is higher than that of the United States, Brazil, China, Turkey, Poland, France, and the Czech Republic. The variant allele G frequency was higher in Germany (79%), India (65%), Italy (75%), Romania (78%), Spain (75%), and Tunisia (61%) than in Saudi Arabia. Ethnic heritage affects a person's vulnerability to certain diseases (36). Therefore, the effect of ethnicity is indicated by the variation in the NER gene XPG exon 15 C>G polymorphism in the Saudi population compared to other populations globally. Research on genetic variations can help identify key risk factors for exposure to contaminants and malignancies, which may have implications for future preventative and detection strategies. Several factors, including ethnic variance, study population heterogeneity, and variations in sample sizes, may contribute to the variations in allelic frequencies found among these investigations. Individual DNA repair SNPs may have a smaller increase or decrease in risk than high-penetrance cancer genes, but because they are highly prevalent in the general population, they may have significant implications for public health. Therefore, epidemiological studies on DNA repair polymorphisms based on ethnicity are crucial (34). To decrease the possibility of false-positive and false-negative outcomes, large and integrated analyses might be preferable. A risk factor in one community may not be applicable in another because the incidence of DNA repair polymorphisms varies by population. The establishment of clinical and epidemiological databases may be based on such research.

Country	Total No.	Mean Age (years), age ±SD	CC (%)	CG (%)	GG (%)	p-value	Variant allele G	Reference
Saudi Arabia	100		18 (18)	50 (50)	32 (32)	Ref.	57	13
USA	219	49.3 ±15.2	127 (58)	80 (37)	12 (5)	<0.001	24	14
Brazil	208		109 (52.4)	74 (35.6)	25 (12)	0.048	30	15
China	176	58.8 ±9.1	78 (44.32)	62 (35.23)	36 (20.4)	0.752	38	16
Czech	532	57.4 ±12.8	356 (66.9)	153 (28.8)	23 (4.3)	<0.001	19	17
France	53		31 (58)	21 (40)	1 (2)	0.013	22	18
Germany	374		18 (4.81)	124 (33.16)	232 (62.03)	<0.001	79	19
India	288		37 (12.84)	129 (44.79)	122 (42.36)	0.132	65	20
Italy	250		15 (6)	94 (38)	141 (56)	0.001	75	21
Korea	311	60.5 ±9.9	90 (28.9)	132 (42.4)	89 (28.6)	0.844	50	22
Poland	100		64 (64)	31 (31)	5 (5)	0.010	21	23
Romania	533		30 (5.6)	173 (32.5)	330 (61.9)	<0.001	78	24
Spain	214	53.9 ±8.4	14 (6.5)	81 (37.9)	119 (55.6)	0.002	75	25
Tunisia	125		18 (14.4)	61 (48.8)	46 (36.8)	0.583	61	26
Turkey	96		43 (44.8)	44 (45.8)	9 (9.4)	0.008	33	27

Table 1: Genotype and allele frequency distributions of *XPG* exon 15(rs17655) gene polymorphisms in various populations and p-values compared to those in the Saudi Arabian population

CONCLUSIONS

To summarize, our study showed that Saudi populations differ from populations around the world in the frequency of the genetic variant of XPG exon 15 C>G. This polymorphism can reduce DRC among Saudi individuals, increase cancer susceptibility, and act as a biomarker for cancer risk. Understanding the prevalence pattern of NER gene polymorphisms may help clinicians provide a more accurate prognosis and help discuss the expected outcomes, risks, and treatment options with patients.

INFORMED CONSENT STATEMENT

Not required as this study has been done through laboratory-based software, such as SPSS 21, for allele and genotype frequency determination and statistical analysis.

AUTHOR CONTRIBUTIONS:

Conceived and designed the study and experiments: MW.

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CONFLICTS OF INTEREST

None.

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Case Report

Genetic Evaluation of a 30-Year-Old Female with Unilateral Renal Agenesis: A Case Study

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ABSTRACT Unilateral renal agenesis (URA) is a congenital anomaly where one kidney fails to develop, often diagnosed in childhood or prenatally. While many cases are asymptomatic, URA is associated with long-term renal risks and potential genetic implications. This study presents the case of a 30-year-old female with incidentally detected URA, evaluating her renal function, genetic background, and clinical implications. Ultrasound confirmed a solitary kidney with no surgical history. Renal function tests were normal. Genetic analysis revealed no pathogenic variants, suggesting a sporadic occurrence. This case highlights the importance of URA diagnosis in asymptomatic adults, emphasizing long-term monitoring and genetic evaluation to guide clinical management and patient counseling.

Keywords: Unilateral Renal Agenesis; Kidney; Genetics.

INTRODUCTION

Renal agenesis is the lack of renal tissue arising from an early embryonic developmental abnormality [1]. Additional congenital defects have occasionally been associated with both unilateral and bilateral renal agenesis. The disorder results from the ureteric bud failing to fuse with the metanephric blastema, therefore absenting the nephron and often the ureter. Rare occurring in fewer than one in 3,000 to 4,000 births, bilateral renal agenesis is connected to severe neonatal morbidity and death resulting from lethal pulmonary hypoplasia brought on by the absence of amniotic fluid. By contrast, 1 per 1,000 live infants have URA, usually linked with a good prognosis due to compensatory enlargement of the solitary kidney [2].

Although the origin of congenital kidney and urinary tract anomalies is unknown, considerable evidence points to a genetic link; family clustering has been noted in certain cases [3]. Diverse mutational mechanisms and molecular pathways suggest that about 20% of people with congenital kidney abnormalities may have an undetected inherited disease. Different congenital kidney and urinary tract abnormalities seem to have a shared genetic basis impacted by polygenic inheritance, cytogenetic abnormalities (e.g., copy number variations), and both autosomal dominant and recessive mutations [5].

In this case, we present a patient with URA, highlighting the role of genetic testing in assessing potential hereditary factors. Whether URA arises in isolation or is linked to mutations in important renal developmental genes including PAX2, RET, EYA1, SIX1, and HNF1B, which are linked with renal abnormalities, is mostly dependent on genetic study [6]. Risk assessment, long-term treatment, and family counseling all depend on an awareness of the genetic foundation of URA. This paper emphasizes the need of including genetic testing into clinical practice to improve diagnosis accuracy and maximize patient treatment.

CASE PRESENTATION

A thirty-year-old lady had a full medical checkup, laboratory testing, and ultrasonic imaging. The patient was observed on an ultrasonic scan to have a solitary kidney without accompanying kidney in situ and no signs of surgical excision or scarring. The patient claims no positive urine problems; she has no renal pathology and no recognized congenital defects. Normal serum electrolytes coupled with normal renal function including serum urea at 3.5mmol/L, creatinine at 74 micromole/L, and an eGFR within normal range were shown by laboratory analysis. as hereditary testing was done to screen for PAX2, RET, EYA1, SIX1, HNF1B, and other genes linked with renal development and congenital abnormalities as renal agenesis is typically linked with genetic problems.

To investigate other extra-renal congenital abnormalities like hearing loss, ear pits, cleft lip or palate, and anosmia that commonly have syndromic associations with renal agenesis, a detailed examination was conducted. The study sought to ascertain if the condition was an independent aberration or a component of some syndromic multi-phenotypic disease, thereby guiding the design of therapy, identification of aetiologic variables, and accurate genetic counseling.

CASE PRESENTATION

Genetic Testing:

A genetic assessment for the possible origins of URA was performed on the patient. The next step was to conduct next generation sequencing (NGS) on the exons of genes associated with kidney development and their abnormalities. This was done through a targeted gene panel including PAX2, RET, EYA1, SIX1, and HNF1B which are well known for their links with renal agenesis and related disorders of the kidney. The NGS technology employed provided unparalleled sensitivity and specificity in detecting pathogenic variants, which made sustentative diagnosis and clinical intervention feasible.
[6].

Clinical Assessment

Tests for renal funciton, such as serum creatnine and other glomerokus filtration estimations were done to see how well the remaning kidney works. Ultrasound was done to assess the kidney's structure and morphology.

RESULTS

Genetic Testing:

Genetic analysis revealed no pathogenic variants or mutations in genes associated with renal development or congenital anomalies. The patient genetic makeup was within the normal range, suggesting that her URA may not have a significant genetic basis.

Medical Evaluation:

The results for serum creatinine levels and the eGFR were within the anticipated values for a single kidney patient, indicating normal kidney function. The ultrasound confirms that a right solitary kidney exists. In the top image, the kidney is identifiable by its bean shape with identifiable echogenic renal cortex and hypoechoic medulla. In the bottom image, the right side labeled "R" is the indicating the right kidney with normal morphology while the left side "EMPTY" indicates no kidney is present at the position of left renal fossa. This ultrasound report correlates with the description of empty left renal fossa which substantiates the presence of single right kidney.

Table 1: Renal Function Tests. The table indicates the renal function tests with all parameters which show the normal condition for all parameters

Test Name	Result	Reference Range
COLOR	YELLOW	YELLOW
Reaction (pH)	6.0	6.0
Specific Gravity	1.015	1.000 - 1.030
Protein	NIL	NIL
Glucose	NIL	NIL
Acetone	negative	NEGATIVE
Nitrite	Negative	NEGATIVE
Bilirubin	Nil	NIL
Urobilinogen	Normal	NIL
RBC	3-5	< 1
Pus cell/HPF	20-25	1 - 5
Epithelial Cells	+++	NIL
Crystals	NIL	NIL
Casts / H.P.F	NIL	NIL
Parasites & Ova	NIL	NIL
Mucus Threads	NIL	NIL
Others	BACTERIA ++ YEAST CELLS +	NIL

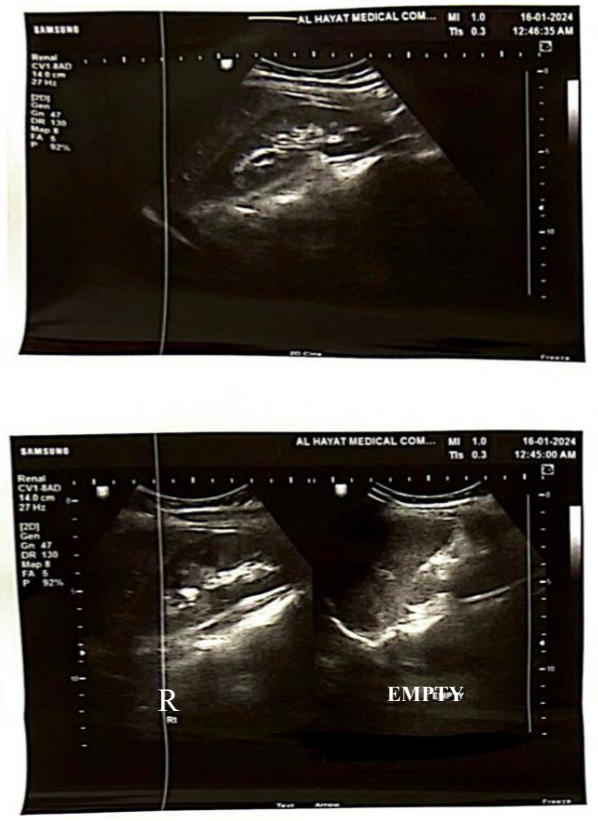

Figure 2: Ultrasound image depicting a solitary kidney with normal morphology and structural integrity. The nephrologist has confirmed that the case is entirely normal, with no abnormalities detected. The abdominal and pelvic ultrasound reveals a normal-sized liver with homogeneous texture, no focal lesions, and normal portal vein (PV), common bile duct (CBD), and biliary tree. The gallbladder has a normal wall thickness with no stones. The right kidney is of normal size, with a preserved corticomedullary differentiation (CMD), normal echopattern, and no evidence of renal gravels or backpressure changes. The left renal fossa is empty.

DISCUSSION

Large pedigrees suitable for linkage analysis of renal agenesis, hypoplasia, and dysplasia are difficult to identify due to the partial penetrance of these conditions, which are influenced by both genetic and environmental modifiers. Furthermore some anomalies, such URA, could be asymptomatic and undetectable without thorough family screening. Locus heterogeneity poses a difficulty in candidate gene investigations, thereby maybe compromising the effectiveness of linkage studies [7].

The lack of harmful mutations in genes linked to renal development in this patient points to URA perhaps being sporadic or idiopathic rather than clearly genetically derived. Although environmental impacts and developmental abnormalities may also be important in those with a normal genetic background, genetic elements contribute to some cases [8]. This emphasizes how urgently more study on non-genetic causes of renal agenesis is needed. Early detection and therapy of probable consequences depend on long-term renal function monitoring even if later life brings possible

hazards related to hypertension, proteinuria, and chronic kidney disease (CKD) [9].

Unilateral renal agenesis is often linked to genetic factors, with mutations in genes like PAX2, RET, EYA1, SIX1, and HNF1B playing a crucial role in kidney development. It is associated with syndromes such as Renal Coloboma Syndrome, Branchio-Oto-Renal Syndrome, Mayer-Rokitansky-Küster-Hauser Syndrome, and VACTERL association, which may present with additional congenital abnormalities [10]. The prevalence of URA-related syndromes varies by region, with consanguinity increasing the risk of inherited renal anomalies. Environmental factors, including maternal diabetes and teratogenic exposures, may also contribute. Given these associations, genetic counseling and targeted genetic testing are essential for diagnosis, prognosis, and reproductive planning [6].

Individuals diagnosed with URA require continuous oversight to maintain renal health and prevent complications such as chronic kidney disease (CKD), proteinuria, and hypertension. Early detection of dysfunction is dependent on regular urinalysis, blood pressure monitoring, and evaluation of renal function. Protective strategies for renal tissues include adequate hydration, a balanced diet, and the avoidance of nephrotoxic medications. Routine ultrasound examinations of the kidneys are essential for identifying structural abnormalities and compensatory hypertrophy. In aging patients, growth monitoring is advisable, and women may require gynecological assessments due to potential links with Müllerian anomalies. In areas with a higher incidence of congenital renal deformities, nephrology monitoring and genetic counseling may be advantageous. Effective intervention and risk reduction depend on the consistent monitoring of renal health, regardless of symptom presentation [2-5].

CONCLUSIONS

This case study highlights the importance of genetic evaluation and medical monitoring in individuals with URA. While some cases may have a genetic basis, others may occur sporadically or have multifactorial etiologies**.** Understanding the genetic and health implications of URA in individuals with normal genetic makeup is crucial for providing personalized care and genetic counseling**.**

AUTHOR CONTRIBUTIONS:

The author solely conceived, designed, and executed the study, including data collection, analysis, and interpretation. The author also drafted, revised, and approved the final manuscript for submission.

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ETHICAL CONSIDERATIONS:

The consent of the case was obtained for this case study and genetic testing.

CONFLICTS OF INTEREST

The author declares no conflict of interest.

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Case Report

Primary Thyroid Tuberculosis: A Case Report from Jazan, Saudi Arabia

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ABSTRACT: Thyroid tuberculosis is a rare disease. Diagnosis can be difficult because the clinical course is not very specific in most cases. Ziehl–Neelsen staining is frequently negative. Epithelioid granulomas and necrosis are the main biopsy findings. The diagnosis can be confirmed using polymerase chain reaction, detection of acid-fast bacilli, or bacteriological cultures.

I report the case of a young patient who presented with anterior neck swelling. Primary thyroid tuberculosis was diagnosed based on the clinical picture and fine-needle aspiration cytology findings. Treatment with a combination of anti-tuberculous chemotherapy was initiated using the WHO category I regimen, and the patient showed an excellent response to treatment. To my knowledge, this is only the second clinical case of primary thyroid tuberculosis to be reported in Saudi Arabia.

In conclusion, tuberculosis of the thyroid gland should always be considered in the differential diagnosis of goiters, albeit being rare. The presence of cold abscesses and discharging sinuses in the midline of the neck raises suspicion of this disease, especially in endemic areas. The clinical presentation, in addition to strong evidence from fine-needle aspiration cytology, is sufficient for the diagnosis of this condition.

Keywords: Thyroid, Tuberculosis, Epithelioid Granuloma, Thyroiditis, Neck Cold Abscess.

INTRODUCTION

The involvement of the thyroid gland with primary tuberculosis is rare, and some eminent pathologists in the nineteenth century believed that the thyroid gland is never affected by tuberculosis. [1, 2]. According to many studies, the incidence of primary thyroid tuberculosis in histologically examined thyroid specimens ranges from 0.4 to 1.15% [3-8]. In a retrospective review of 527 thyroid biopsies over 20 years, Al-Mulhim et al. reported two patients with tuberculous thyroiditis in eastern Saudi Arabia (approximately 0.4 %) [3]. My search cited only one clinical case of thyroid tuberculosis reported by Alshareef et al. in the western region of Saudi Arabia [9].

The diagnosis of thyroid tuberculosis remains challenging due to the rarity of this condition and the nonspecific nature of its clinical presentation. It can be confused with other thyroid conditions, such as multinodular goiter, granulomatous thyroiditis, Hashimoto's thyroiditis, bacterial abscesses, and thyroid malignancies [10]. However, establishing a diagnosis is crucial to avoid unnecessary thyroid surgery and delays in treatment. Antituberculous medications are well tolerated and highly effective, especially when diagnosed early.

CASE DESCRIPTION

Patient Information: A 26-year-old male patient presented to our outpatient department complaining of anterior neck swelling for one year. He reported a thick yellow discharge from the swelling seven months before presenting to the hospital, which stopped spontaneously after two weeks. He denied any history of fever but reported having trouble swallowing, which started a few weeks before visiting our clinic. No weight loss, chronic cough, or any other symptoms were reported. The patient had close contact with a patient with tuberculous lymphadenitis.

Clinical Findings: On examination, there was a non-tender, firm, and partially cystic swelling in the anterior aspect of the neck, which moved with deglutition There was a transverse scar of a healed sinus on the left side of the swelling with seropurulent discharge from its medial aspect (Figures 1 and 2). The systemic examination was normal.

Diagnostic Assessment: Thyroid ultrasound revealed enlargement of both thyroid lobes with a heterogeneous non-

uniform echo pattern and complex variable-sized solid and cystic nodules with multiple foci of microcalcification involving both thyroid lobes and the isthmus. Peripheral hypervascularity and enlarged deep cervical lymph nodes with preserved shapes were observed. Retrosternal extensions were not observed. Computed tomography (CT) of the neck confirmed the ultrasound findings (Figure 3). Thyroid function tests showed normal T3 and T4 levels but significantly elevated TSH levels. Fine-needle aspiration cytology revealed several epithelioid cells, one multinucleated giant cell with scattered neutrophils, and a spectrum of lymphoid cells. Follicular epithelial cells were scant, and the background showed necrotic debris. Both Ziehl–Neelsen staining and culture results were negative. Polymerase chain reaction was unavailable at the time. Chest radiography revealed clear lungs bilaterally.

Therapeutic Intervention: Tuberculous thyroiditis was diagnosed based on the clinical presentation and the findings of fine-needle aspiration cytology (FNAC). The patient was administered a combination of rifampicin 600 mg, isoniazid 300 mg, pyrazinamide 1500 mg, and ethambutol 800 mg daily for the first two months of treatment, followed by a four-month period of rifampicin and isoniazid.

Follow-up and Outcomes: The patient complied with the treatment and did not experience any adverse drug reactions. The patient responded well, and the sinuses healed completely within two months of treatment initiation.

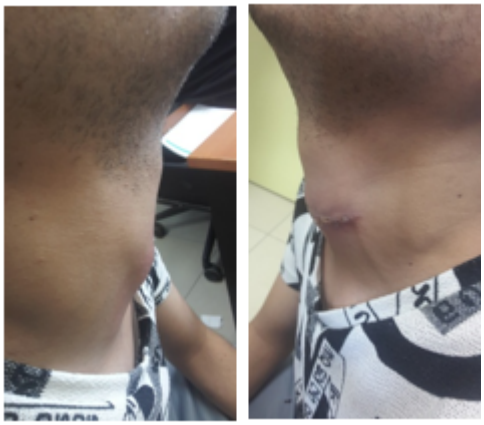

Figure 1: Right and left side pictures of the patient showing thyroid enlargement and scar

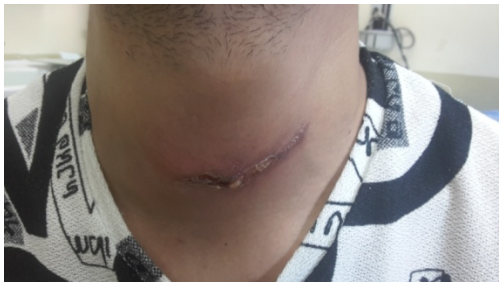

Figure 2: Anterior view showing thyroid enlargement, scar, and a partially healed sinus

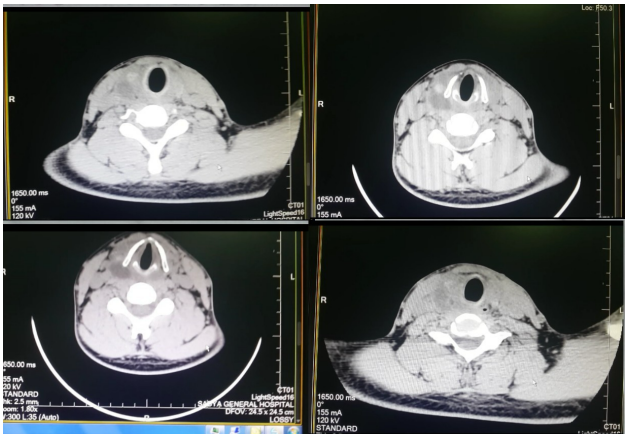

Figure 3: CT scan showing heterogeneous enlargement of the thyroid gland with some deep cervical lymph node enlargement

DISCUSSION

Thyroid tuberculosis is an extremely rare condition, even where the disease is endemic [8]. Diagnosis is challenging because there is no specific clinical presentation, and it presents in various ways [11]. It can present as a thyroid nodule or cold abscess, with or without systemic symptoms [12]. Pressure symptoms such as dysphagia, hoarseness of the voice, and dyspnea have all been reported to be associated with tuberculous thyroiditis. This has led to the suspicion of malignancy in some cases [13, 14]. It can present as acute infectious thyroiditis or follow a subacute course mimicking other forms of thyroiditis, such as De Quervain's thyroiditis [15]. Euthyroid, hyperthyroid, and hypothyroid states were observed in association with this condition [14,15]. Failure to make a diagnosis or misdiagnosis can lead to unnecessary thyroid surgery [16].

The initial assessment of thyroid disease involves ultrasound, thyroid function tests, and FNAC. Ultrasound is non-invasive and very informative in the hands of experienced sonographers. It is the most sensitive method for detecting thyroid enlargement, nodules, cysts, cyst-like lesions, and enlarged cervical lymph nodes [17]. However, it cannot differentiate between different pathologies because the findings are nonspecific, especially in thyroid tuberculosis. Kang et al. described a predominantly anechoic, well-defined lesion with internal echoes in one patient and a heterogeneous, predominantly anechoic lesion with irregular margins in another [18]. Heterogeneous, hypoechoic, irregular thyroid lesions, whether single or multiple, have been described in many cases [19]. When CT is performed in such cases, it usually confirms ultrasound findings and may detect other enlarged cervical lymph nodes. Although thyroid function tests are important in the assessment of thyroid diseases, they do not help diagnose tuberculous thyroiditis, as we have seen earlier. FNAC remains the mainstay of diagnosis for this condition. It is a minimally invasive and cost-effective procedure that can be

performed in outpatient settings. Cellular material can be obtained for microscopy, ZN staining, culture as well as polymerase chain reaction (PCR). Ultrasound guidance and novel techniques such as universal sample processing can be used to increase the yield of FNAC. The presence of epithelioid granulomas and caseous necrosis is consistent with the diagnosis of tuberculous thyroiditis [4]. The diagnosis can be confirmed using ZN staining, especially in doubtful cases. However, the sensitivity of AFB and culture can be low (39–80%), and some samples are both AFB- and culture-negative [20]. PCR can be particularly useful for the confirmation of the diagnosis [20]. Different combinations of these methods should be used to clarify the diagnosis. Our patient showed ultrasonographic and cytological findings similar to those previously described. Diagnosis was made based on clinical presentation and cytological findings, and treatment was initiated accordingly.

CONCLUSIONS

Tuberculosis of the thyroid gland should always be considered a possibility in the differential diagnosis of goiters, although it is rare. The presence of cold abscesses and discharging sinuses in the midline of the neck raises suspicion of this disease, especially in endemic areas. The clinical presentation, in addition to strong evidence from fine-needle aspiration cytology, is sufficient to diagnose this condition.

INFORMED CONSENT STATEMENT

Written informed consent was obtained from the patient for the publication of this case report and the accompanying images. Ethical approval for the publication of this case was waived by the ethics committee of the Faculty of Medicine, Jazan University.

DATA AVAILABILITY STATEMENT

The images and data discussed in this report are available upon reasonable request from the corresponding author.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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