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MAJOR ENVIRONMENTAL FACTORS CONTRIBUTING TO CONGENITAL SCOLIOSIS

Koloyan Z. A.[2], Aleksanyan A. A. [3], Yeritsian S. A.[1,2*], Magardichian M.[4], Koloyan G. A.[1,2], Aebi M.[5]

[1.]Department of Pediatric Orthopedics, Traumatology and Spine Surgery, Yerevan State Medical University, Yerevan, Armenia
[2.]Department of Pediatric Orthopedics and Traumatology and Spine Surgery,Wigmore Clinic, Yerevan, Armenia
[3.]Department of Microsurgery and Plastic Surgery, Yerevan State Medical University, Yerevan, Armenia
[4.]-University of California Los Angeles, Los Angeles, CA, USA
[5.]-Spine Unit, University of Bern, Bern, Switzerland

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Abstract

This review aims to identify, track, and analyze the environmental factors affecting the high incidence of Congenital Scoliosis in the Republic of Armenia. The information gathered from the literature came from MEDLINE, Embase, and CENTRAL ranging from the studies done from 1952-2019, which included twenty-six animal studies and thirteen human studies. Regarding congenital scoliosis, the possible environmental factors can be classified as: hypoxia, Carbon Monoxide, maternal diabetes, valproic acid, hyperthermia, twinning, reduction of fetal movements, vitamin A deficiency, boric acid, and alcohol. Comprehension of the causes of Congenital Scoliosis establishes a crucial step into designing preventive measures. Although rare in published literature, it is the notion of orthopedic surgeons specializing in vertebral pathologies, that in certain geographic locations such as the Republic of Armenia, the rates of congenital scoliosis are much higher, leading to significant healthcare burden. In order to further grasp the epidemiology and pathophysiology of congenital scoliosis, more clinical research is required in the form of large data sets to reach a higher level of evidence for causation. Based on the twenty-six animal and thirteen human models, there was insufficient causation for the human studies. Based on the literature review conducted, there were no prospective/cohort studies on the effects of environmental factors and a lack of adequate controls on the potential causation of congenital scoliosis. A study will be conducted using a questionnaire and medical records to determine whether congenital scoliosis could be potentially affected by environmental factors to determine a causation analysis.

Keywords: *Congenital Scoliosis, hypoxia, diabetes mellitus, pregnancy*

Introduction

Congenital scoliosis (CS) is defined as a spinal curvature of more than 10 degrees in the coronal plane caused by vertebral malformations present at birth [*Grivas TB, et al., 2010*]. The reported incidence of CS is estimated to be anywhere from ten to one hundred cases per 100,000 births [*Hedequist D, Emans J, 2007, Passias PG, 2017*,], and annually *one surgery is* attributed to CS taking place per 100,000 population [*von Heideken J, 2018*].

Congenital scoliosis is now classified based on the morphology of the underlying vertebral malformations which can be either formation defects (e.g., wedge vertebra, hemivertebra), segmentation defects (e.g. block vertebra, unilateral unsegmented bar), or a combination of both (e.g. unilateral unsegmented bar with contralateral hemivertebra) [*Hedequist D, Emans J, 2007, Marks DS,*

Address for Correspondence:
Sevan A Yeritsian, M.D.
Department of Pediatric Orthopedics, Traumatology and Spine Surgery, Yerevan State Medical University after M. Heratsi, 2 Koryun Street, Yerevan 0025, Armenia
Tel: +37498440252
E.mail: sevan.eritsian19@gmail.com

Qaimkhani SA 2009; Kawakami N, 2009]. These malformations appear to arise from the failure of early developmental processes.

The vertebral column derives from presomitic mesoderm which is a group of mesenchymal cells situated on both sides of the neural tube. During somitogenesis, beginning from the rostral end, presomitic mesoderm repetitively undergoes segmentation at regular intervals (4–5 hours in humans) resulting in the formation of somites. The ventral parts of somites give rise to the mesenchymal sclerotome, which encompasses the progenitor tissues of vertebrae and ribs [*Dequéant ML, Pourquié O, 2008*, *Scaal M, 2016*]. The periodic process of somite formation is coordinated by the "clock and wavefront" mechanism originally proposed by Cooke and Zeeman [*Cooke J, Zeeman EC, 1976*]. According to this model, a molecular network of Notch, Wnt/b-catenin, and Fibroblast Growth Factor (FGF) signaling pathways induces bands of gene expression in presomitic mesoderm, moving from caudal to rostral end in an oscillatory pattern (the "clock"). At the rostral end of presomitic mesoderm the opposing gradients of FGF and Wnt (caudal to rostral), and retinoic acid (RA) (rostral to caudal) signaling pathways establish a maturation determination front (the "wavefront") which moves caudally with each oscillation. The interaction of the two components of the "clock and wavefront" model activates the expression of certain genes (most importantly Mesp2), forming a region of gene expression, the prepattern of the future somite [*Pourquié O, 2011*].

The disruption of somitogenesis, particularly due to alterations in underlying molecular signaling mechanisms leads to various congenital vertebral malformations (CVM) [*Pourquié O, 2011*]. Two CVM associated syndromes: spondylocostal dysostosis and Alagille syndrome, are found to occur due to monogenic mutations (*DLL3, MESP2, LFNG, HES7,* and *JAG1,* respectively) involved in the Notch signaling pathway [*Giampietro PF et al., 2008*, *Giampietro PF, 2012*].

To overcome it is possible, due to the uniting the knowledge and will of all doctors in the world

Despite the striking recent findings on embryogenesis and pathophysiology of vertebral malformations [*Pourquié O, 2011*], the etiology of congenital scoliosis remains incompletely understood. Both genetic and environmental factors appear to be associated with CS. Heterozygous null mutations in TBX6, S225N variant allele of DLL3, haploinsufficiency of HES7 or MESP2 are associated with congenital scoliosis in humans [*Giampietro PF et al., 2008*, *Sparrow DB et al., 2012, Wu N et al., 2015*]. In contrast to the plethora of genetic research, the environmental impact on the development of CS remains under-investigated.

The current paper intends to elaborate on the environmental causes and risk factors of congenital scoliosis by reviewing the published literature to date. The insights gained from this review will allow future research to specifically investigate the most likely environmental causes.

RESULTS

We identified thirty-nine studies investigating potential risk factors for CS. The characteristics of the studies according to the investigated risk factors/causes of CS, and study design can be found in tables 1 and 2, respectively. We identified twenty-six animal studies and thirteen human studies on CS. All the animal studies were maternal exposure models at various stages of embryonic development. Overall, the studies investigated various environmental exposures as risk factors for CS. The human studies were case reports, case series, reviews, or surveillance studies of small patient populations (encompassing one to twenty-seven patients with CS). As evident from the tables, we were unable to identify large or prospective human studies on potential risk factors and causes of CS.

DISCUSSION

HYPOXIA

Six studies were investigating the role of hypoxia in the development of CS [*Ingalls TH et al.,, 1952*, *Ingalls TH et al., 1953, Ingalls TH, Curley FJ, 1957*, *Murakami U KY, 1963, Rivard CH, 1986, Sparrow DB et al., 2012*]. Intrauterine hypoxia can be caused by various maternal, placental, and fetal conditions, and is accordingly categorized into

preplacental (e.g., high-altitude, maternal cardiovascular, hematological diseases, acute respiratory infections), uteroplacental (e.g., placental insufficiency, preeclampsia), or post placental (e.g., fetoplacental perfusion defects, fetal cardiovascular, genetic disorders) subgroups. Prolonged in-utero hypoxia is known to induce fetal intrauterine growth restriction, multiorgan failure, asphyxia, premature birth, and is associated with increased perinatal mortality [*Hutter D et al., 2010*]. In addition, chronic hypoxia caused by lack of HbF, maternal heavy cigarette smoking, or living at very high altitude has been linked to fetal congenital malformations (e.g., limb and digital defects, orofacial clefting) [*Webster WS, Abela D, 2007*].

In experimental animal models, it has been shown that hypoxic or anoxic episodes during pregnancy can be teratogenic, commonly causing limb, heart, Central Nervous System (CNS), and genitourinary defects, orofacial clefting, midfacial hypoplasia [*Webster WS, Abela D, 2007*]. In murine models, maternal short-term high altitude hypoxia simulation (by lowering the air pressure in the chamber) during the early gestational period resulted in vertebral malformations in the offspring. Among these malformations hemivertebrae, the fusion of vertebrae and ribs, fragmented vertebral bodies were frequently observed, indicating the disruption of segmentation and formation processes [*Ingalls TH et al., 1952, Ingalls TH et al., 1953, Ingalls TH, Curley FJ, 1957, Murakami U KY, 1963, Rivard CH, 1986*]. In mice, the critical period of vertebral development most susceptible to hypoxic injury was between the ninth and tenth days of gestation [*Ingalls TH et al., 1952*]. The incidence and severity of malformations were directly associated with the extent of hypoxia and the duration of exposure [*Ingalls TH, Curley FJ, 1957*]. Among mice exposed to hypoxia, a clear correlation was observed between the time of exposure and the craniocaudal level of vertebral defects: the later the exposure, the more caudal the levels of the spine were affected [*Ingalls TH, Curley FJ, 1957, Murakami U KY, 1963*].

Increased incidence of skeletal anomalies

Table 1.

Identified Studies According to Investigated Risk Factors / Causes

Investigated Risk Factors / Causes	References
Hypoxia	[*Ingalls TH et al., 1952*] [*Ingalls TH TH et al., 1953*] [*Ingalls TH, Curley FJ, 1957*] [*Sparrow DB et al., 2012*] [*Rivard CH, 1986*] [*Murakami U KY, 1963*]
Carbon Monoxide	[*Loder RT et al., 2000*] [*Farley FA et al., 2001*] [*Farley FA 2006*] [*Alexander PG, Tuan RS, 2003*]
Vitamin A	[*Li Z et al., 2012*]
Diabetes	[*Holmes LB, 2011*] [*Ewart-Toland A et al., 2000*]
Valproic acid (VPA)	[*Downing C et al., 2010*] [*Vorhees CV, 1987*] [*Menegola E et al., 1996*] [*Ardinger HH et al., 1988*] [*Bantz Mew, 1984*]
Hyperthermia	[*Shiota K, 1988*] [*Weiss J, Devoto SH, 2016*] [*Breen JG 1999*] [*Harrouk WA et al., 2005*]
Fetal immobility	[*Rolfe RA 2017*] [*Levillain A et al., 2019*] [*Panter KE 1990*] [*Panter KE et al., 1990*]
Twinning	[*Sturm PF et al., 2001*] [*Pool RD, 1986*] [*Ogden JA, Southwick WO, 1978*] [*Kaspiris A et al., 2008*] [*Greenwood D, Bogar W, 2014*] [*Holmes LB, 2011*] [*Greenwood D, Bogar W, 2014*]
Alcohol	[*Tredwell SJ et al., 1982*] [*Schilgen M, Loeser H, 1994*]
Boric Acid	[*Harrouk WA et al., 2005*] [*Wéry N et al., 2003*]
Zinc	[*Hickory W et al., 1979*]
Mycophenolate mofetil (MMF)	[*Perez-Aytes A et al., 2017*]
NO	[*Alexander PG et al., 2007*]
Almokalant	[*Sköld AC et al., 2001*]

TABLE 2.

Identified Studies According to Study Design

Design	References
Animal Exposure Studies	
Chick Embryo Model	[*Rolfe RA et al., 2017*] [*Li Z et al., 2012*], [*Alexander PG, Tuan RS, 2003*], [*Alexander PG et al., 2007*]
Murine Model	[*Sparrow DB et al., 2012*] [*Ingalls TH et al., 1952*], [*Ingalls TH et al., 1953*], [*Ingalls TH, Curley FJ, 1957*], [*Murakami U KY, 1963*], [*Rivard CH, 1986*], [*Loder RT, et al., 2000*], [*Farley FA et al., 2001*], [*Farley F 2006*], [*Downing C et al., 2010*], [*Menegola E et al., 1996*], [*Shiota K, 1988*]
Rat model	[*Li Z et al., 2012*], [*Vorhees CV, 1987*], [*Menegola E et al., 1996*], [*Breen JG et al., 1999*], [*Harrouk WA et al., 2005*], [*Wéry N et al., 2003*], [*Sköld AC et al., 2001*], [*Hickory W et al., 1979*]
Goat model	[*Panter KE et al., et al., 1990*], [*Panter KE et al., 1990*]
Zebrafish	[*Weiss J, Devoto SH, 2016*]
Human Studies	
Case Reports & Case Series	[*Bantz Mew, 1984*], [*Schilgen M, Loeser H, 1994*], [*Sturm PF et al., 2001*], [*Ogden JA, Southwick WO, 1978*], [*Kaspiris A et al., 2008*], [*Greenwood D, Bogar W, 2014*], [*Pool RD, 1986*], [*Cho W et al., 2018*]
Larger Patient Population Reviews	[*Ardinger HH et al., 1988*], [*Perez-Aytes A et al., 2017*], [*Tredwell SJ 1982*], [*Ewart-Toland A et al., 2000*], [*Holmes LB, 2011*]

was observed among four out of five strains of mice emphasizing the effect of the strain variation on the expression of defects and suggesting an interaction between genetic factors and hypoxic stress [*Ingalls TH et al., 1953*]. It has been demonstrated that mild hypoxia near the threshold of susceptibility of somitogenesis significantly increases the frequency and severity of vertebral malformations in genetically predisposed mice [*Sparrow DB et al., 2012*]. These findings support the multifactorial nature of CS and the roles of both genetic and environmental etiologies.

In a murine model, hypoxic exposure during the critical period of vertebral development diminished the glycosaminoglycan synthesis in notochord-sclerotome complexes, suppressed the cell proliferation and differentiation, thereby leading to defective primordial cartilage formation which develops into a malformed vertebra after ossification [*Rivard CH, 1986*]. It was concluded that short-term hypoxia interrupts somitogenesis due to perturbations in FGF signaling, the latter being one of its main signal transduction pathways; and that disruptions of Wnt and Notch1 pathways occur as secondary events [*Sparrow DB et al., 2012*].

We suggest that any factor inducing intrauterine hypoxia during the early stages of pregnancy might disrupt the somitogenesis, resulting in vertebral malformations in the fetus. There may be an interplay between genetic predisposition and hypoxic insult.

CARBON MONOXIDE

Our search revealed four studies indicating the role of carbon monoxide in the etiology of CS [*Loder RT, Hernandez MJ, Lerner AL, et al., 2000, Farley FA, Loder RT, Nolan BT, et al., 2001, Alexander PG, Tuan RS, 2003, Farley FA, Hall J, Goldstein SA, 2006*]. Carbon monoxide (CO) is an odorless, colorless toxic gas resulting from incomplete combustion of carbon-based compounds (e.g., wood, petrol, coal, natural gas, kerosene). The common sources of CO are motor vehicle exhaust, improperly working heating and cooking devices, indoor burning of biomass fuels, and fire in fireplaces [*WHO, 2010*]. Another major source of CO exposure is tobacco smoke which contains 4% CO on average. The toxic effect of CO is linked to its 200 times higher affinity to hemoglobin compared to oxygen. Carbon monoxide binds with he-

moglobin forming carboxyhemoglobin, resulting in reduced tissue oxygenation [*Longo LD, 1977*]. Acute CO poisoning is not uncommon and causes non-specific symptoms (e.g., headache, dizziness, nausea, fatigue) in mild cases, and if severe, can lead to coma, respiratory failure, and death [*Kao LW, Nañagas KA, 2005*]. Maternal CO exposure during pregnancy has been associated with congenital malformations (e.g., microcephaly, micrognathia, limb defects) in the offspring [*Alexander PG, Tuan RS, 2010*].

Loder et al. [*Loder RT et al., 2000*] and Farley et al. [*Farley FA et al., 2001*, *Farley FA et al., 2006*] have developed an experimental mural model demonstrating that maternal CO exposure at critical periods of pregnancy produces congenital scoliosis in the offspring. Short-term exposure to doses of CO that are clinically applicable to humans (200, 400, and 600 *ppm*) produced anomalies similar to those caused by simulation of high-altitude hypoxia. Radiographic examination revealed various congenital malformations (e.g., hemivertebrae, wedge vertebrae, absent and fused vertebrae, fused ribs) affecting all spinal regions [*Loder RT et al., 2000*]. There was a direct correlation between CO dose and the incidence of vertebral malformations. In CD-1 and DBA/1J strains of mice, the most susceptible period of CO exposure occurred at 9th and 9.5th days of gestation, respectively [*Loder RT et al., 2000*, *Farley FA et al., 2001*]. Compared to the correlation found in the high-altitude hypoxia model [*Ingalls TH, Curley FJ, 1957*, *Murakami U KY, 1963*], no evident connection between the period of CO treatment and the level of malformations was observed [*Loder RT et al., 2000*]. Chronic exposure to lower levels of CO (50, 100, and 200 *ppm)* during the first eleven days of gestation did not produce significant spinal anomalies in CD-1 mice [*Farley FA et al., 2001*]. Observation of the exposed offspring at near-maturity confirmed that earlier described vertebral malformations developed into congenital scoliosis [*Farley FA et al., 2006*].

Another experimental model of CO exposure was conducted by Alexander et al. [*Alexander PG, Tuan RS, 2003*]. Exposure of chick embryos to 1000 *ppm* of CO during early (thirty-six to fifty-four hour) development resulted in multiple spinal anomalies, including fused vertebrae, absent vertebral elements, absent ribs, and mild to severe scoliosis. The authors suggested that even in absence of apparent neural tube malformations, CO exposure primarily affects the neural tube and interrupts inductive signaling from it, leading to suppressed *Paraxis* gene expression and somite dysmorphogenesis. The possible mechanisms of CO-induced teratogenesis can be mediated by its property as a hypoxia-inducing agent as well as due to its excessive action as an endogenous signaling molecule [*Alexander PG, Tuan RS, 2010*].

Vitamin A deficiency

We identified one article investigating the role of vitamin A deficiency as a cause of CS [*Li Z et al., 2012*]. Vitamin A deficiency (VAD) remains a significant public health concern in developing countries and it is prevalent in pregnant women and in children due to the vitamin's availability from a restricted range of nutriments [*Haider BA, Bhutta ZA, 2011*].

Retinoic acid (RA), the active form of vitamin A, is an important signaling molecule involved in human as well as other mammalian embryonic development. The physiological transformation of vitamin A to RA is mediated by retinaldehyde dehydrogenases. Biosynthesised RA regulates gene expression at the transcriptional level by binding to two families of nuclear receptors called ring sideroblasts and retinoid X receptors[*Rhinn M, Dolle P, 2012*]. RA has an important role in many physiological processes, such as vision, immune responses, cell differentiation [*Whatham A et al., 2008, Pino-Lagos K et al., 2011, Mendoza-Parra MA et al., 2011*] and participates in the formation of various organs (the CNS, heart, eye, skeleton, forelimb buds, lungs, pancreas, and genitourinary tract) [*Duester G, 2008*]. It has been demonstrated that RA signaling is essential for optimal left-to-right patterning during somitogenesis, and that disruption of such signaling leads to asymmetric axial development [*Vermot J, Pourquié O, 2005, Vermot J, 2005, Kawakami Y et al., 2005, Sirbu IO, Duester G, 2006*].

VAD produces multiple anomalies among humans and experimental animals [*Clagett-Dame M, Knutson D, 2011*]. In a rat model, VAD most frequently caused ocular and genitourinary defects, less frequently leading to diaphragmatic, cardiovascular, pulmonary anomalies [*Wilson JG et al., 1953*].

In the offspring of rats maternal diet deficient in vitamin A during gestation produced multiple skeletal anomalies, including cranial abnormalities, reduced sizes of axial and extremity skeleton, vertebral anteriorizations, absence or fusions of ribs, and sternal malformations [*Clagett-Dame M, 2008, See AWM et al., 2010 Li Z, Shen J, Wu WK, et al., 2012*,]. Although no gross spinal anomalies were present, radiographic examination revealed thoracic congenital vertebral malformations, including hemivertebrae and fused vertebrae in 14% of the VAD group offspring compared to 0% in controls. It was demonstrated that vitamin A deficiency suppressed the mRNA expression of retinaldehyde dehydrogenases and ring sideroblasts in the livers and vertebral bodies of neonatal rats, suggesting interruption of the RA signaling pathway [*Li Z et al., 2012*].

Overconsumption of dietary vitamin A or RA derivatives has also been linked to teratogenesis [*Ross S et al., 2000*]. Maternal use of 13-cis-RA has been associated with high levels of spontaneous abortions and neonatal malformations, such as craniofacial, cardiac, thymic, and CNS defects [*Lammer E et al., 1985*].

These findings suggest that any alterations in the RA signaling might be associated with skeletal abnormalities. Particularly, vitamin A deficiency or aberrant vitamin A transformation into RA can lead to congenital scoliosis in humans.

HYPERTHERMIA

There were four articles exploring the possible role of hyperthermia in the development of CS [*Shiota K, 1988 et al., 1999, Harrouk WA et al., 2005, Weiss J, Devoto SH, 2016*]. It is known that hyperthermic episode during embryonic development can be teratogenic or can cause abortion or stillbirth. Humans, as well as other homeothermic species, are frequently exposed to elevated body temperatures (e.g., due to febrile illness, strenuous physical activity, environmental heat). It has been demonstrated that body temperature elevations of 1.5-2.5°C were teratogenic in different animal species [*Edwards MJ, 1986*].

Maternal hyperthermia over 38.9°C during the early stages of pregnancy has been associated with multiple anomalies, including anencephaly [*Miller P et al., 1978*], meningomyelocele [*Chance P et al., 1978*], encephalocele [*Fisher N, Smith D, 1981*], other functional and structural CNS abnormalities, and facial dysmorphogenesis [*Fraser FC et al., 1982*]. Hyperthermia has also been demonstrated to be teratogenic in various experimental animal species, producing defects in different organ systems (e.g., CNS, heart, neural tube, eyes), analogous to those observed in human fetuses exposed to maternal fever during pregnancy [*Edwards MJ, 2006*].

K. Shiota demonstrated that short-term (5-15min) maternal exposure to temperatures 3.5-4.5°C higher than normal during the critical period (8.5 day) of murine development frequently produced skeletal malformations, such as defective or absent vertebral bodies and arches, bent or fused ribs. Increased axial skeletal variations including irregular or extra sternebrae, cervical or lumbar ribs and split vertebral arches were present [*Shiota K, 1988*]. It has been demonstrated that both the duration and the degree of heat are in direct relationship with the teratogenicity of hyperthermia. Relatively small elevation (2-3°C) in body temperature in rats at gestational day 10 also produced malformed vertebrae and ribs in the offspring [*Kimmel CA et al., 1993*].

Hyperthermic insult resulted in temporarily increased cell death rate and/or altered proliferation in CNS, optic cup, neural tube, somites as well as in presomitic mesoderm [*Breen JG et al., 1999*], thereby possibly contributing to anomalies in developing organs (particularly in the axial skeleton).

Weiss et al. demonstrated that heat shock and osmotic shock administered during development affected the segmentation process and resulted in somite border anomalies in zebrafish embryos. Both exposures affected the somitogenesis in a dose-dependent pattern by increasing the incidence and severity of anomalies. Exposure to heat or hyperosmolarity resulted in a delayed effect on the segmentation process; borders were defective in somites that were formed after, rather than during the exposure, indicating that the alteration took place in the presomitic mesoderm situated in the determination front of segmentation at the moment of exposure. Both heat shock and osmotic shock produced similar changes in the segmentation process, suggesting that various environmental stressors might affect somitogenesis through a common pathway. The possible targets of these stressors

might be the genes involved in the regulatory network of somitogenesis, such as the components of "the segmentation clock", or "the wavefront" [*Weiss J, Devoto SH, 2016*].

It has been demonstrated that exposure to various factors including hypoxia, hyperthermia, and hyperosmolarity results in endoplasmic reticulum (ER) stress due to the accumulation of unfolded proteins in the ER lumen [*Kitamura M, 2013*, *Enserink JM, 2015*]. ER stress leads to unfolded protein response (UPR), thereby regulating ER homeostasis, or in failure to do so, activates the apoptotic pathways [*Walter P, Ron D, 2011*]. Shi et al. [*Shi H et al., 2016*] suggested that any stressor that activates UPR during pregnancy suppresses FGF signaling, thus possibly resulting in congenital defects. Although there is a lack of epidemiological data on CS among humans exposed to maternal hyperthermia, it would be plausible to conclude from the above, that exposure to hyperthermia (e.g., fever) might play a crucial role in the etiology of spinal malformations, particularly in CS.

Valproic acid

We identified five articles supporting the role of valproic acid in the etiology of CS [*Bantz Mew, 1984, Vorhees CV, 1987, Ardinger HH et al., 1988, Menegola E et al., 1996, Downing C et al., 2010*]. In addition to its wide use as an anti-epilepsy drug, VPA is also used for the treatment of bipolar disorder [*Peselow ED et al., 2016*], prevention of migraines [*Kinze S et al., 2001*] and has shown promise as an anticancer agent [*Heers H et al., 2018*].

The teratogenicity of antiepileptic drugs (AEDs) is well known [*Hill DS et al., 2010*]. Furthermore, there is an increased risk of congenital malformations in groups of neonates exposed to the combination of AEDs compared to monotherapy groups. In one report 9% of infants whose mothers were administered AEDs during pregnancy had congenital abnormalities. Although carbamazepine was more frequently used, valproic acid (VPA) produced malformations with higher incidence compared to other AEDs. Valproic acid was associated with an increased risk of cardiac defects, hypospadias, spina bifida, orofacial cleft, and other congenital abnormalities [*Wide K et al., 2004*].

Bantz reported a case of congenital scoliosis in a girl whose mother received valproic acid (250 *mg tid*) throughout her pregnancy. The child developed vertebral defects of the 1st, 2nd, and 3rd lumbar vertebrae accompanied by thoracolumbar scoliosis, among other malformations [*Bantz ME, 1984*]. Ardinger described three cases of spinal anomalies, two of which were lumbosacral meningomyelocele and the third was a "butterfly" thoracic vertebra. In all three cases, the mothers were using VPA in combination with other AEDs during pregnancy [*Ardinger HH et al., 1988*].

The teratogenic profile of valproic acid has been reproduced in experimental animal models. Maternal VPA administration at days seven to eighteen of gestation caused skeletal anomalies in a dose-dependent manner in the rat offspring. Although the doses of VPA (150-200 *mg/kg*) that establish drug plasma levels within the human therapeutic range were found to be mildly teratogenic, higher doses (400 *mg/kg*) frequently produced skeletal defects, including ectrodactyly, fused, and wavy ribs, short or missing tales, and split or missing vertebrae [*Vorhees CV, 1987*]. In contrast, VPA was more embryo-lethal and teratogenic in mice than in rats. The doses ranging from 150 to 300 *mg/kg* significantly increased the incidence of axial skeletal malformations, including additional, fused, or absent vertebrae, fused ribs, and asymmetric sternum in mice [*Menegola E et al., 1996*]. In two different strains of mice (B6 and D2) maternal VPA exposure at the ninth day of gestation produced different patterns of skeletal malformations. While malformations of digits and vertebrae were more common in B6 fetuses, rib anomalies were more frequently observed in D2 strain. After reciprocal mating of the two strains and the same maternal exposure, the genetically homogenous F1 offspring were examined. The incidence of vertebral malformations was higher in F1 offspring of B6 mothers than in F1 offspring of D2 mothers, thereby underlining the role of maternal genotype and/or uterine environment in VPA susceptibility. It was concluded that both maternal and fetal genetic factors are involved in VPA teratogenesis [*Downing C et al., 2010*].

It has been demonstrated that valproic acid inhibits histone deacetylases (HDACs), among other known mechanisms of action [*Phiel CJ, Zhang F, Huang EY, et al., 2001*, *Gottlicher M, 2001*]. The teratogenic effects of VPA are associated with

HDAC inhibition and resulting histone acetylation [*Menegola E et al., 2005*, *Eikel D, Lampen A, Nau H, 2006*]. By controlling gene expression, HDACs regulate many important cellular functions, including proliferation, differentiation, and apoptosis [*Marks PA et al., 2000*]. Histone deacetylases also control bone formation and skeletal development [*Westendorf JJ, 2007*]. Interestingly, other HDAC inhibitors, such as apicidin, MS-275, butyric acid, boric acid, and sodium salicylate also produce axial skeletal defects, particularly vertebral malformations [*Di Renzo F et al., 2007a, b, 2008*].

Considering the wide application of VPA and its well-known teratogenic profile, it would be plausible to establish further epidemiological data on its association with congenital scoliosis.

REDUCTION OF FETAL MOVEMENTS

We found 4 studies investigating the association of fetal immobility and CS [*Panter KE et al., 1990a, b, Rolfe RA, et al., 2017, Levillain A, et al., 2019*]. Fetal movements begin to occur at about 7 gestational weeks in humans [*De Vries JI, Fong BF, 2006*] and play a crucial role in fetal development, and particularly in the formation of the musculoskeletal system [*Kalampokas E et al., 2012*, *Nowlan NC, 2015*]. The decrease in fetal motility causes multiple congenital contractures (arthrogryposis), and in severe cases, fetal akinesia deformation sequence [*Witters I et al., 2002*]. Hypoplasia of vertebral bodies accompanied by scoliosis has been described in patients with FADS [*Bisceglia M et al., 1987*]. There is a high prevalence of scoliosis with various patterns of curves observed among patients with arthrogryposis [*Yingsakmongkol W, Kumar SJ, 2000*]. Drummond et al. reported 50 cases of arthrogryposis, 7 of which were associated with congenital scoliosis [*Drummond DS, Mackenzie DA, 1978*], and Fletcher et al. described 6 cases of co-occurring arthrogryposis and congenital cervical scoliosis [*Fletcher ND et al., 2010*].

In animal models, it has been demonstrated that fetal movements during embryogenesis play an important role in spine formation. In a chick embryo model prolonged rigid paralysis induced by decamethonium bromide (a depolarizing neuromuscular blocking agent that eliminates dynamic muscle forces) during embryonic development resulted in significant alterations in spinal sagittal curvatures, vertebral segmentation, and shape, while flaccid paralysis brought on by pancuronium bromide (a non-depolarizing neuromuscular blocker eliminating both static and dynamic muscle forces) only produced mild defects in vertebral shape. Rigid paralysis at earlier stages of embryogenesis produced more severe malformations [*Rolfe RA et al., 2017*]. It was shown that short-term immobilization at 3rd and 4th days of avian embryonic development had major consequences on spinal curvature, vertebral shape, and segmentation, while paralysis at the 5th day was more critical for rib formation. Based on the observation that this critical period coincides with sclerotome cell migration and differentiation [*Christ B et al., 2000*], it was hypothesized that dynamic muscular activity plays a key role in the development of ventral sclerotome, which is the progenitor of vertebral bodies and intervertebral disks [*Levillain A et al., 2019*].

Piperidine alkaloids (e.g., anabasine, coniine, lobeline) are known ligands of nicotinic acetylcholine receptors, and their action is mainly mediated by disruption of cholinergic neurotransmission [*Green BT et al., 2013*]. Piperidine alkaloids inhibit fetal movements and cause teratogenesis in animal species. Panter et al. demonstrated that administering plants containing piperidine alkaloids (Conium *maculatum*, Nicotiana *glauca*, Lupinus *formosus*) to goats during 30-60th days of gestation inhibits fetal movements and produces cleft palate and arthrogryposis in the offspring. In the affected subjects vertebral wedging and concomitant scoliosis were observed among other abnormalities [*Panter K et al., 1990a, b*, *1990*].

Fetal immobility plays a possible role in the etiology of CS. Embryonic paralysis induced by cholinergic disruption appears to be a suitable model for congenital spinal abnormalities exhibited by vertebral wedging and fusion of vertebral elements, as demonstrated in both avian [*Rolfe RA et al., 2017*, *Levillain A et al., 2019*] and mammalian [*Panter K et al., 1990a, b*, *1990*] models.

Decrease in fetal motility can be caused by a myriad of fetal disorders, such as neurological, muscular, connective tissue diseases, or can be induced by fetal environmental anomalies, such as restricted intrauterine space (e.g., due to twinning, oligohydramnios, amniotic bands, uterine malfor-

mations), compromised fetal blood supply and maternal disorders (e.g., maternal diabetes, myasthenia gravis, multiple sclerosis, infections, trauma, toxins or drugs) [*Kowalczyk B, Feluś J, 2016*].

Piperidine alkaloids can be found in tobacco [*Green BT et al., 2013*]. Cigarette smoking is the most likely scenario of environmental human exposure to agents responsible for embryonic paralysis (i.e., alkaloids), and therefore carries an additional potential role in the pathogenesis of CS (see the paragraph on "CO").

Maternal diabetes

Two studies identified the role of maternal diabetes in the etiology of CS [*Ewart-Toland A et al., 2000, Holmes LB, 2011*]. Maternal pregestational diabetes (PGD) is associated with a twofold to ninefold increased risk of congenital malformations in the offspring. Although PGD is associated with abnormalities in almost all organ systems, the cardiovascular, central nervous, and skeletal systems are the most affected [*Gabbay-Benziv R, 2015*]. One of the most characteristic congenital defects caused by diabetes is the caudal regression syndrome, which is 200-600 times more prevalent in the offspring of diabetic compared to healthy mothers [*Wender-Ozegowska E et al., 2005*].

Although less frequently, gestational diabetes (GD) was also found to increase the risk of congenital defects [*Balsells M et al., 2012*]. GD was significantly associated with holoprosencephaly, upper/lower spine/rib defects, renal and urinary system anomalies [*Martínez-Frías ML et al., 1998*].

Aberg et al. demonstrated that maternal PGD and GD were associated with a 4.8-fold and 2.6-fold higher risk of spine malformations, respectively [*Åberg A et al., 2001*]. Martinez-Frias et al. observed that 17% (5/29) of vertebral and rib malformations were attributed to maternal diabetes [*Martinez-Frias ML, 1994*]. Similarly, in a surveillance study of 206,244 infants, 27 cases of hemivertebrae were documented [*Holmes LB, 2011*]. The most frequent associated condition was maternal insulin-dependent diabetes (in 5 out of 27 cases). A case of hemivertebra and 5 cases of fused vertebrae were observed in 21 infants with craniofacial anomalies born to diabetic mothers [*Ewart-Toland A et al., 2000*].

Maternal diabetes is known to increase the production of reactive oxygen species in the primordial tissues of malformed organs [*Alexander PG, Tuan RS, 2010*]. Elevated reactive oxygen species-activity leads to oxidative stress, which activates JNK1/2 mediated pro-apoptotic signaling pathways leading to diabetic embryopathies. Activation of JNK1/2 induces ER stress leading to UPR [*Gabbay-Benziv R, 2015*]. As already discussed in the section on hyperthermia, UPR activation disrupts FGF signaling, thereby possibly leading to congenital vertebral malformations [*Sparrow DB et al., 2012, Shi H et al., 2016*].

Boric acid

In 2 articles boric acid has been discussed as a possible contributor to CS [*Wéry N et al., 2003, Harrouk WA, Wheeler KE, Kimmel GL, et al., 2005*]. Boric acid (BA) is an inorganic agent used in manufacturing processes and consumer products. In medicine, it continues to be used as an antiseptic agent [*Wéry N et al., 2003*]. In animal models, BA has been shown to be teratogenic. In murine models, maternal exposure to 1000 *mg/kg* doses of BA at GD8 produced a significantly higher incidence of axial skeletal malformations in the offspring (e.g., fused vertebrae and ribs, hemivertebrae, homeotic transformations) [*Di Renzo F et al., 2007*]. Coexposure to hyperthermia and BA had an additive effect on the frequency of segmentation abnormalities (e.g., fused or deformed ribs, fused or asymmetric vertebrae), and demonstrated synergism regarding the reduction of vertebral numbers [*Harrouk WA et al., 2005*]. Wery et al. demonstrated that BA induced axial skeletal anomalies associated with altered expression of *hox* genes [*Wéry N et al., 2003*]. Boric acid exposure was shown to alter histone acetylation specifically in the developing somites thereby suggesting that BA produces axial skeletal malformations by suppressing HDAC activity, in a manner similar to VA and other HDAC inhibitors [*Di Renzo F et al., 2007*].

Twinning

We identified 7 articles discussing the association of twinning and CS [*Holmes LB, 2011 et al., 1978, Pool RD et al., 2001, Kaspiris A et al., 2008, Greenwood D, Bogar W, 2014, Cho W et al., 2018*]. Twinning is associated with a relatively high risk of congenital defects compared to singleton gestations [*Corsello G, Piro E, 2010*]. It is suggested to

result in circulatory compromise and fetal hypoxia (e.g., twin-twin transfusion, steal phenomenon) [*Maier R et al., 1995*], and thereby is a possible risk factor in the pathogenesis of congenital vertebral anomalies.

Several cases of congenital scoliosis have been described among monozygotic and dizygotic twins and triplets [*Ogden JA et al., 2014*]. Among 27 infants with hemivertebrae 2 cases were associated with twinning (only one of each pair of twins were affected) [*Holmes LB, 2011*]. In some cases, only one child of the monozygotic pairs exhibited CS [*Pool RD, 1986*, *Cho W et al., 2018*] or if both were affected, different patterns of deformity were observed [*Sturm PF et al., 2001*, *Kaspiris A et al., 2008*]. This additionally supports the suggestion that CS is not exclusively genetic and that extrinsic environmental factors play a crucial role in its etiology.

ALCOHOL

There were 2 studies investigating the possible role of maternal alcohol consumption in the etiology of CS [*Tredwell S et al., 1982*, *Schilgen M, Loeser H, 1994*]. Maternal use of alcohol during pregnancy causes a broad range of anomalies termed fetal alcohol spectrum disorders. Particularly, in severe cases, it leads to fetal alcohol syndrome which is characterized by facial dysmorphogenesis, growth retardation, and CNS disorders [*Warren KR et al., 2011*]. Fetal alcohol syndrome has been associated with the Klippel-Feil anomaly [*Schilgen M, Loeser H, 1994*], a congenital syndrome characterized by a triad of short neck, low posterior hairline, and limited range of neck movements due to cervical segmentation defects [*Tracy MR et al., 2004*]. In a review of 50 cases, Tredwell et al. reported that 53% (19) of patients with FAS exhibited segmentation defects (two and more fused vertebrae) in the cervical spine similar to those found in Klippel-Feil syndrome [*Tredwell SJ et al., 1982*].

Based on these findings and the observation that Klippel-Feil syndrome is accompanied by CS in more than 50% of cases [*Tracy MR et al., 2004*], we suggest that alcohol consumption during pregnancy might play a role in the etiology of CS.

Other Associations

A number of other environmental factors have been implicated in the etiopathogenesis of CS [*Hickory W et al., 2001, Alexander PG et al., 2007, Perez-Aytes A et al., 2017*]. Feeding rats with a moderately Zinc deficient diet (1.3 *ppm*) during their 3rd to 15th days of gestation produced skeletal abnormalities (e.g., malformations of ribs, vertebrae, and long bones) in the offspring. Fifty percent of fetuses in the Zinc deficient group exhibited hemivertebrae [*Hickory W et al., 1979*].

Alexander et al. demonstrated both the increase of NO levels by administration of NO donor sodium nitroprusside and the decrease of NO production by nitric oxide synthase inhibitor methyl-L-arginine (L-NMMA) disrupted normal axial skeletal development in the chick embryo. In viable embryos among several sites, significant changes were observed in somites, segmental plate, neural tube, which are the progenitors of the vertebral column. Exposure to L-NMMA reduced apoptotic activity in tissues responsible for axial skeletal development. Contrariwise, SNP exposed embryos demonstrated increased apoptosis in the same structures. Coadministration of exogenous CO modulated the teratogenic effects of L-NMMA or sodium nitroprusside treatments, suggesting the existence of physiological NO-CO signaling pathways. It was hypothesized that disruption of this signaling plays an important role in NO-induced skeletal dysmorphogenesis [*Alexander PG et al., 2007*].

Almokalant is a class III antiarrhythmic drug that acts by blocking the potassium channels (I_{Kr}-blocker). In the offspring of rats, a single dose administration of Almokalant on different days of gestation (throughout days 10-14) produced time-specific skeletal abnormalities (e.g., fusion, misalignment, ossification defects of vertebrae and ribs) similar to those caused by short term hypoxia. It was suggested that all I_{Kr}-blockers can lead to teratogenesis by induction of embryonic arrhythmia and resulting reoxygenation damage [*Sköld AC et al., 2001*].

Mycophenolate mofetil (MMF) is an immunosuppressant frequently used after organ transplantations and in the treatment of autoimmune conditions. Use of MMF during the first trimester of pregnancy is associated with a high risk of drug-specific embryopathy characterized by a typical triad of defects (orofacial clefting, otic and ocular anomalies). Vertebral malformations, such as

hemivertebrae and bifid vertebrae were observed in 12.5% of cases of MMF embryopathy [*Perez-Aytes A et al., 2017*], suggesting a potential role for the pharmaceutical to be a risk factor for CS.

Conclusion

There is scant data on environmental causes and risk factors for congenital scoliosis. Most of the current knowledge emanates from animal maternal exposure studies, and most of the human research is based on small populations and is descriptive at best. Furthermore, animal studies provide insufficient evidence on causation in humans. Additionally, there appear to be no prospective/cohort studies and a lack of adequate controls, thereby shadowing any potential for causation analysis. Not infrequently many authors attribute any features of CVM to CS, thereby contributing to sample heterogeneities. It is our impression that CVM should not always be documented as CS.

Throughout our review we were able to identify the following environmental exposures as potential risk factors and/or causes for CS, namely, hypoxia, CO, maternal diabetes, valproic acid, hyperthermia, twinning, reduction of fetal movements, vitamin A deficiency, boric acid, and alcohol appear as viable candidates as major risk factors.

Understanding the causes of CS provides important insights into designing preventive measures. Despite its infrequent occurrence in the published literature [*Hedequist D et al., 2017*], it is the impression of both Armenian and foreign orthopedic surgeons specializing in vertebral pathology, that in certain geographic locations such as the Republic of Armenia, the rates of CS are much higher, leading to significant healthcare burden. In order to further understand the epidemiology and pathophysiology of CS, more clinical research is required in larger volumes to reach a higher level of evidence.

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ACUTE EXACERBATION OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE WITH POLYMORPHIC PHAGOCYTIC CELLS

Rahmaputra Y.D., Subkhan M.*, Mochtar N.M.*

Faculty of Medicine, Muhammadiyah University of Surabaya, Surabaya, Indonesia

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Abstract

Chronic obstructive pulmonary disease is a progressive non-communicable disease due to the chronic inflammation response. An acute exacerbation is a weighting of the symptoms experienced by the patient before. Acute exacerbations of Chronic obstructive pulmonary disease are thought to be associated with acute inflammation involving pro-inflammatory cells. Bangil Hospital data in 2019, Chronic obstructive pulmonary disease patients reached 2928 and 388 exacerbation events. Study aims to analyze the correlation between acute exacerbation of chronic obstructive pulmonary disease with polymorphic phagocyte cells in the Emergency Department of Bangil Hospital, Indonesia.

This was a cross-sectional retrospective study. We collected the patient who came to the emergency room with acute exacerbation event and recorded the number of times the patient experiences an acute exacerbation event and the results of a complete blood lab polymorphic phagocyte cell, including basophils, neutrophils and eosinophils from the medical record. Data analysis in this study used the Spearman method to determine the correlation between Chronic obstructive pulmonary disease exacerbation and polymorphic phagocyte cells.

Chronic obstructive pulmonary disease exacerbations 1-5 times were obtained in 54 patients. The patients were 41 males (75.9%) and 13 females (24.1%). The chronic obstructive pulmonary disease patients were 46-65 years (n=31, 52.4%), >65 years (n=21, 38.9%), and 26-45 years (n=2, 3.7%). Patients who had moderate symptoms were 34 patients (61.1%), severe symptoms 7 patients (12.9%), and mild symptoms 13 patients (24.1%). Statistical test results showed an association of acute exacerbation of chronic obstructive pulmonary disease with basophils (p=0.018), neutrophils (p=0.001), and eosinophils (p=0.0012).

This study concludes there is a correlation between the incidence of acute exacerbation and chronic obstructive pulmonary disease with polymorphic phagocyte cells.

Keywords: *chronic obstructive pulmonary disease, acute exacerbations, neutrophil, basophil, eosinophil.*

Introduction

Chronic obstructive pulmonary disease (COPD) is a non-communicable, preventable disease, and it is a public health problem in Indonesia [*Rosyid A, Maranatha D, 2018*]. Based on WHO data, in 2002, COPD ranks 5th as a cause of death globally, and it is estimated that in 2030 it will be third worldwide after cardiovascular disease and cancer. The Asian Pacific COPD Round Table Group estimates that moderate to severe COPD patients in Asia Pacific countries in 2006 had reached 56.6 million people with a prevalence of 6.3%. Meanwhile, the number of COPD patients in Indonesia is estimated to be 4.8 million, with a majority of 5.6% [*PDPI, 2016; Agusti A, Vogelmeier CF., 2018*]. The significant risk of COPD is cigarette smoking which leads to the development of COPD [*Kwon N et al., 2013*].

Indonesia has the highest number of smokers and ranks first in Southeast Asia [*Ismail I et al., 2020*]. Smokers spanned through different social classes, status, gender, and age. The major causes of smoking behavior are the availability of cigarettes everywhere in the country [*Pribadi E, Devy S, 2020*]. The prevalence of smokers in Indonesia

Address for Correspondence:
Muhammad Subkhan, MD;
Nur Mujaddidah Mochtar, MD
Faculty of Medicine Muhammadiyah University of Surabaya, Jalan Sutorejo No 59, Surabaya 60113, Indonesia
Tel.: (+6231)3811966
E-mail: msubkhan74@gmail.com, sayamujaddidah@gmail.com

in 2010 was 34.7%, with the highest prevalence in Central Kalimantan province, Indonesia with 43.2% and the lowest in Southeast Sulawesi with 28.3% [*PDPI, 2016*]. There was a high smoking prevalence in the age group of 25-64 years with a range of 37-38.2%, and 18.6% in the population aged 15-24 years who smoked every day. The prevalence of male and female smokers was 16 times higher in men with a value of 65.9% compared to the value for women of 4.2%, showing that men dominated the number of smokers in Indonesia [*Ummah F et al., 2020*]. Elderly smokers have a 50% risk of developing COPD [*Lundbäck B et al., 2003*]. In addition to the dose of cigarettes, types of cigarettes also have a chance of increasing lung function abnormalities. Secondhand smoke also has the same risk level as active smokers for developing COPD. Based on RISKESDAS data in 2013, East Java, Indonesia, had a COPD prevalence of 3.6%. Based on Bangil Hospital, Indonesia, there were 2059 patients diagnosed with COPD and 282 patients with acute COPD exacerbation in 2018. In 2019, there was an increase in patients to 2928 patients diagnosed with COPD, but there was a decrease in COPD patients' acute exacerbation to 106 patients [*Indonesia Ministry of Health, 2013*].

Bacteria mostly are the cause of acute exacerbation of COPD patients at Arifin Rachmad Hospital in Riau, Indonesia. Gram-negative bacteria are the most common cause than gram-positive bacteria [*Martantya R et al., 2014*]. COPD is a preventable and treatable chronic lung disease characterized by persistent and usually progressive airway limitation [*Hariyono R et al., 2019*]. It is associated with an excessive chronic inflammatory response to the airways and lung parenchyma due to harmful gases or particles. Moreover, it can result in tissue damage to the airway. As a result of the inflammatory response, the body releases pro-inflammatory mediators such as TNF-α, IL-6, and IL-8, which will attract CD8 + Tc1 cells, macrophages, B lymphocytes, and polymorphic phagocyte cells consisting of neutrophils, eosinophils, and basophils. This inflammatory process will cause pathological changes that cause narrowing of the airways, leading to air trapping and limited airflow. Acute exacerbation of COPD is an acute event characterized by worsening of the patient's respiratory condition, such as shortness of breath and cough with or without sputum from day to day compared to before. Exacerbations and comorbid play a role in the severity of COPD [*Indonesia Ministry of Health, 2013; Martantya R et al., 2014*].

This study was conducted by performing a polymorphic phagocyte cell count test in COPD patients who came to the emergency department during acute exacerbations. Acute exacerbation of COPD was chosen because it has characteristic symptoms compared to stable COPD. in Previous research journals on COPD, an increase in neutrophils and no increase in eosinophils or basophils [*Martantya R et al., 2014*], whereas, in previous research journals on COPD exacerbations, there was an increase in neutrophils, but not eosinophils [*Juwariyah J et al., 2017*]. There was no significant and statistically significant correlation between acute exacerbation of COPD with basophils, eosinophils, and neutrophils in both research journals [*Martantya R et al., 2014; Juwariyah J et al., 2017*]. This study aims to determine the correlation between the incidence of acute exacerbation of COPD with polymorphic phagocyte cells consisting of basophils, neutrophils, and eosinophils.

Material and methods

This was a quantitatives research using a retrospective cross-sectional design. This study aims to determine and analyze the correlation between the incidence of acute exacerbation of COPD and polymorphic phagocyte cells. It is hoped that the correlation between acute exacerbation of COPD and polymorphic phagocyte cells consists of 3 types, namely basophils, eosinophils, and neutrophils. In this study, the population to be studied was an acute exacerbation of COPD patients who came to the Bangil Hospital Emergency Department, Indonesia, from 2018 to 2019. The research sample was taken according to inclusion using the simple random sampling method and obtained a sample of 54 patients. Data collection was carried out from December 2019 to

To overcome it is possible, due to the uniting the knowledge and will of all doctors in the world

January 2020. The independent variable in this study was COPD patients with acute exacerbations, and the measurement results were the number of times they experienced exacerbations with an ordinal data scale. In contrast, the dependent variable was the phagocyte cells present in the complete blood cell examination, namely basophils, neutrophils and eosinophils, with measurement results in the form of percentages and interval scales.

In this study, the data collection procedure was carried out after obtaining ethical permission from the Faculty of Medicine ethics team, Muhammadiyah University of Surabaya, Indonesia and internal ethics permission from the ethics team of the Bangil Hospital, Indonesia. The medical record data that has been obtained is then analyzed using the univariant correlation test to see the value of the frequency distribution of each variable and the spearman bivariant correlation to determine and assess the correlation between COPD exacerbations and polymorphism phagocyte cells. The correlation level criteria were assessed from a sig >0.05, and the levels ranged from almost no correlation to perfect correlation. Processing and data analysis were carried out using the 25th edition of the Statistical Package for the Social Sciences application to obtain the results and conclusions of this study.

Results

Based on table 1, among the 54 patients in the study, there were 41 males (75.9%) and 13 females (24.1%). The majority of COPD patients in this study were 46-65 years old. The majority of patients had moderate symptoms (61.1

Table 2 reveals that the correlation value between the exacerbation of COPD and basophil polymorphic phagocyte cells is 0.321. This shows a positive correlation between the incidence of exacerbation of COPD and basophil polymorphic phagocytic cells. The higher the incidence of exacerbation of COPD, the higher the basophil cell levels. There was a significant correlation between the exacerbation of COPD and polymorphic basophil phagocyte cells (p=0.018).

The correlation value between the exacerbation of COPD and the neutrophil polymorphic phagocyte cells was -0.433, as shown in Table 2. This

Table 1.

Characteristics of respondents

Variable	n	%
Sex		
Man	41	75.9%
Woman	13	24.1%
Age		
26-45 years	2	3.7%
46-65 years	31	57.4%
> 65 years	21	38.9%
Symptoms of exacerbation		
Mild Symptoms (1 Symptom)	13	24.1%
Moderate Symptoms (2 Symptoms)	34	63.0%
Severe Symptoms (3 Symptoms)	7	12.9%
Total	54	100.0%

Table 2.

Correlation between chronic obstructive pulmonary disease exacerbation and basophil, neutrophil and eosinophil cell levels

Exacerbation Incident	Cell Levels (%)	Correlation	P
basophil cell levels			
1	0.67		
2	0.95		
3	0.40	0.321	0.018
4	1.25		
5	1.10		
neutrophil cell levels			
1	81.70		
2	71.44		
3	73.60	-0.433	0.001
4	58.95		
5	70.20		
eosinophil cell levels			
1	1.04		
2	2.12		
3	4.30	0.339	0.012
4	9.60		
5	8.10		

indicates a negative correlation between the incidence of exacerbation of COPD with polymorphous neutrophil phagocyte cells. The higher the incidence of exacerbation of COPD, the lower the levels of neutrophil cells. There was a significant correlation between the exacerbation of COPD and neutrophil polymorphic phagocyte cells (p=0.001).

Table 2 shows that the correlation value between the exacerbation of COPD and eosinophil polymorphic phagocyte cells is 0.339. This shows a positive correlation between the incidence of exacerbation of COPD with eosinophil polymorphic phagocyte cells. The higher the incidence of exacerbation of COPD, the higher the levels of eosinophil cells. There was a significant correlation between the incidence of COPD exacerbations and the polymorphic eosinophil phagocyte cells (p=0.012).

Discussion

This study analyzed the correlation between acute exacerbation of COPD and polymorphic phagocyte cells consisting of 3 types: basophils, eosinophils, and neutrophils. The results showed that there was a correlation between the incidence of exacerbation of COPD with polymorphic phagocyte cells, both basophils, neutrophils, and eosinophils. Basophil cell levels tend to increase along with the increasing incidence of exacerbation. In previous studies, it was found that the average count of basophils in patients with COPD did not increase, but the theory states that in people who have a bacterial infection, the count of basophils can increase [*Martantya R et al., 2014*]. Another study shows that decreased levels of eosinophils and basophils affect COPD patients' prognosis [*Xiong W et al., 2017*]. Basophils are circulating granulocytes that respond to allergic stimuli by migration and accumulation at sites of inflammation. Basophils act as phagocytosis, effector cells, and memory cells against allergens by producing lipid mediators and cytokines that can affect blood vessel extravasation and stimulate effector cluster of differentiation 4 (CD4) T cells directly to play a role in tissue remodelling. Cross-linking of FcεRI with the immunoglobulin E (IgE) -antigen complex will cause basophil degranulation and mediators' release, especially histamine [*Bain B, 2014; Murray J et al., 2016*].

Neutrophil cell levels tend to decrease with an increasing incidence of exacerbation. The study results found that the neutrophil levels in the incidence of exacerbation were 1 to 5 times. The theory of Lockwood states that segmented neutrophils increase in response to inflammation and bacterial infection [*Lockwood W, 2020*]. In previous studies [*Martantya R et al., 2014; Juwariyah J et al., 2017*], it was found that there was an increase in neutrophil levels in acute exacerbation of COPD and COPD patients. However, there are differences with other studies' results [*Palange P et al., 2006; Rumora L et al., 2008; Shivanand K et al., 2012*] which found that COPD patients had a normal basophil, eosinophil, neutrophil, lymphocyte, and monocyte count. Increased neutrophil counts in COPD patients with comorbid infections may result from a response to airway inflammation, systemic inflammation, and also due to co-infectious diseases in the patient [*Furutate R et al., 2016*]. A study conducted by *Kurtipek E*. and co-authors explained that examination by looking at the levels of neutrophils and lymphocytes in acute exacerbated COPD patients tend to increase and is positively correlated with C-reactive protein testing, addition neutrophil, and lymphocyte examinations are parameters that are easy to measure, and economic costs but with a relatively high diagnostic accuracy [*Kurtipek E et al., 2015*]. When neutrophilia occurs in response to inflammation, it is sometimes accompanied by morphological changes such as toxic granulation, Döhle bodies, and vacuolization. Also, neutrophils may be associated with the presence of mucous hypersecretion [*Xiong W et al., 2017; Agusti A, Vogelmeier CF., 2018*].

The level of eosinophil cells tends to increase along with the increasing incidence of exacerbation. there is an increase in eosinophils in acute exacerbation of COPD [*Papi A et al., 2006; Bafadhel M et al., 2011*]. Besides, administration of corticosteroids can reduce the exacerbation of patients and improve clinical symptoms [*Woods J et al., 2014*]. Previous study stated that the average eosinophil level was >2%, an increase in eosinophil levels was associated with a response to corticosteroid therapy in patients [*Singh D et al., 2014*]. The correlation between eosinophils and corticosteroid therapy was also found in the study of Wei X. and colleagues, which stated that there was a high number of eosinophils in stable COPD pa-

tients who responded to oral and inhaled corticosteroid administration [*Wei X et al., 201*]. There is an informative correlation between pre-therapy blood eosinophil levels and the frequency of COPD exacerbations [*Pavord I et al., 2016*]. In another study it was found that there was a decrease in eosinophil activity after being hospitalized, but it was not statistically significant [*Juwariyah J et al., 2017*]. Increased number of eosinophils in the airways is a pathological feature of asthma. In COPD, it is suspected that eosinophils play a role in the incidence of acute exacerbations and are often associated with poor lung function measurement results, including airway hyperresponsiveness. Drugs that suppress airway eosinophils, including corticosteroids, anti-IgE, and anti-IL-5, are generally effective at lower exacerbation levels [*Murray J et al., 2016*].

This study has limitations. First, it only used medical record instruments in which the researcher is not directly involved in the patient's diagnosis. Second, the sampling did not match one of the exclusion criteria because the field conditions were different. Third, the lack of sample size is also a limitation of this study.

Conclusion

There is a correlation between the incidence of exacerbation of COPD with polymorphic phagocyte cells, both basophils, neutrophils, and eosinophils. Basophil and eosinophil cell levels tend to increase along with the increasing incidence of exacerbation. However, neutrophil cell levels tend to decrease with an increasing incidence of exacerbation.

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NEUTROPHIL-TO-LYMPHOCYTE RATIO AND RENAL FUNCTION IN HYPERTENSIVE CRISIS PATIENTS

Hartono R.T.D.[1*], Purwanto A.[1], Pangarso D.C.[1], Ludfi A.S.[2]

[1]Department of Emergency, Citra Husada Hospital, Jember, Indonesia
[2]Department of Internal Medicine, Citra Husada Hospital, Jember, Indonesia

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Abstract

Background: *The incidence of hypertensive crisis patients worldwide has been estimated at 1-2% of the population. The objective of this study was to evaluate the correlation between neutrophil-to-lymphocyte ratio and renal function in the acute state of hypertensive crisis.*

Methods: *The study was conducted in Citra Husada hospital in Jember, East Java, Indonesia, including 167 consecutive patients admitted to the hospital between January and December 2019 to diagnose hypertensive crisis. The neutrophil-to-lymphocyte ratio was counted as neutrophil count (/μL) divided by lymphocyte count (/μL). The estimated glomerular filtration rate (ml/min/1.73 m^2) was calculated with the Modification of Diet in Renal Disease equation.*

Results: *Hypertensive crisis proportion was lower in male (40.7%, 68 patients) than females (59.3%, 99 patients). The hypertensive emergency incidence was higher (92.2%, 154 patients) with cerebral events as the most frequent target organ damage (64.1%, 107 patients). neutrophil-to-lymphocyte ratio was significantly lower in hypertensive emergency patients (p=0.033), while estimated glomerular filtration rate was significantly higher in hypertensive emergency patients (p=0.046). neutrophil-to-lymphocyte ratio and estimated glomerular filtration rate correlation was significant (p=0.036) with a very weak score (r=-0.163) and coefficient of determination 0.266. A decrease of estimated glomerular filtration rate of 26.6 % will follow each increase in neutrophil-to-lymphocyte ratio.*

Conclusion: *This study suggested a relationship between the increasing neutrophil-to-lymphocyte ratio with decreasing estimated glomerular filtration rate in hypertensive crisis patients. Further studies are needed to evaluate whether the increase in neutrophil-to-lymphocyte ratio was an acute or chronic disease process.*

Keywords: *. eGFR, hypertensive crisis, hypertensive emergencies, hypertension urgencies, NLR*

Introduction

Hypertension is the most prevalent chronic medical condition afflicting people globally [*Varounis C et al., 2017*]. High blood pressure (BP) is a highly prevalent ailment, and its repercussions (including heart disease, stroke, and kidney failure) are significant public health problems [*Salim H et al., 2020*]. Hypertension contributes as a risk factor for the leading causes of death in Indonesia: stroke and ischemic heart disease [*Centers for Disease Control and Prevention, 2018*]. Hypertension comorbid was one of the causes of coronary heart disease (CHD). High blood pressure would cause direct trauma to the walls of the coronary arteries. Therefore, it facilitated coronary atherosclerosis (coronary factors) [*Saputri F et al., 2020*]. The incidence of hypertensive crisis patients around the world has been estimated at 1-2% of the population [*Chakraborty S, 2017*]. In 2004, hypertension was the third most prevalent condition seen in outpatient clinics in Indonesia. By 2006, it had risen to the second position (4.67 percent). According to risk factor surveys for cardiovascular disease (CVD), the prevalence of hypertension has increased to 13.6 percent in men and 16 percent in women in Indonesia [*Sulistiawati S et al., 2020*].

Hypertensive crisis consists of hypertensive

Address for Correspondence:
Rahma Tansy Dea Hartono, MD
Department of Emergency, Citra Husada Hospital, Jember, Indonesia
Jl. Teratai No.22, Patrang, Jember 68117, Indonesia
Tel.: +6281327619500
E-mail: rahmatansydea@gmail.com

emergency (HE) and hypertensive urgency (HU) [*Chakraborty S, 2017*] are acute life-threatening condition [*Derhaschnig U et al., 2014*]. Over the last year, hypertensive patients have experienced a decrease in production due to inability to work (4%) and 28.2 percent sick leave [*Sulistiawati S et al., 2020*]. HE and HU are a condition of severe hypertension in the presence of target organ damage [*Lukito A et al., 2019*]. The target organs include the brain, retina, large arteries, and kidneys [*Unger T et al., 2020*]. This condition can lead to hypertensive encephalopathy, hemorrhagic and non-hemorrhagic stroke, acute coronary syndromes, pulmonary edema, aortic dissection, renal failure, sympathetic crises, and eclampsia. HU is the isolated elevation of BP without the involvement of target organs with the evidence of clinical, laboratory, or instrumental evidence [*Paini A et al., 2018*]. Systolic blood pressure (SBP) >179 *mmHg* or diastolic blood pressure (DBP) >109 *mmHg* is considered hypertensive crisis [*Varounis C et al., 2017*].

The pathophysiology of hypertensive crises is unknown. This event is thought to be initiated by a sudden increase in vascular resistance caused by humoral vasoconstrictors [*Derhaschnig U et al., 2014*]. BP mechanism of self-regulation hypertensive crisis occurs when the brain, heart, and kidneys are unable to maintain stable blood flow. Mechanical stress is caused by autoregulatory dysfunction in the vascular bed and blood flow, which results in an abrupt increase in blood pressure and systemic vascular resistance [*Varounis C et al., 2017*]. Ischemia and vasoactive mediators release lead injury to endothelial. The renin-angiotensin system is activated, resulting in increased vasoconstriction and the generation of proinflammatory cytokines, such as IL-6 [*Haas A, Marik P, 2006*].

Kidneys are one of organs affected by hypertension, and malignant hypertension can cause decrease in renal function [*Derhaschnig U et al., 2014*]. On the other hand, hypertension places a mechanical strain on the glomerulus, which is closely correlated with chronic kidney disease (CKD) progression. Therefore, CKD and high blood pressure often occur simultaneously, characterized by increased blood pressure with decreased kidney function resulting in poor clinical outcomes [*Thaha M et al., 2019*]. Globally, the prevalence of CKD is growing. The prevalence of CKD is estimated to be between 40–60 cases per million people in poor nations. In Indonesia, nephrology centers estimated a prevalence of CKD of between 200 and 250 cases per million population [*Suprapti B et al., 2019*].

Renal function can be evaluated by the laboratory testing of creatinine and estimated glomerular filtration (eGFR) ratio [*Derhaschnig U et al., 2014*]. An increase will follow the decrease in eGFR in CKD in inflammation characterized by increased cytokines, such as IFN, TNFa, IL-1, IL-6, and IL-10. Surge in cytokines is caused by a disruption of the elimination of these cytokines in the kidneys, repeated infections, increased oxidative stress, uremic conditions, metabolic acidosis also affects the increase in inflammatory conditions [*Hertanto D et al., 2019*]. Additionally, hypertensive patients, particularly those with pre-existing severe renal disease, may see an increase in creatinine [*Derhaschnig U et al., 2014*].

Microinflammation is a significant component of CKD and has a significant role in the pathophysiology of CVD complications, as well as protein-energy deficiency (PED) and mortality [*Thaha M, Widiana I, 2019*]. The inflammatory process can be assessed from increased leukocytes and their subtypes. One of the hallmarks investigated regarding cardiovascular risk is the neutrophil-to-lymphocyte ratio (NLR) [*Sevencan N, Ozkan A, 2018*]. The neutrophil is a polymorphonuclear mature granular leukocyte that has an affinity to the immune complex and phagocytosis. The lymphocyte is one specific immune system and has specific antigens, which are its ligands [*Azikin A et al., 2018*]. NLR is a marker of cardiac or non-cardiac disorders and a predictor of mortality in patients with CVD. NLR has been linked to inflammatory indicators such as IL-6 or high sensitivity-CRP (hs-CRP), as well as endothelial dysfunction and cardiovascular risk in individuals with CKD [*Yoshitomi R et al., 2019*]. Other studies also

To overcome it is possible, due to the uniting the knowledge and will of all doctors in the world

state that hs-CRP and NLR have correlated with kidney functions such as eGFR, cystatin c, and blood urea nitrogen (BUN) [*Rochmanti M et al., 2020*]. IL-6 is also one of the most prominent pro-inflammatory cytokines that can be found in CKD patients. Various studies have shown that IL-6 levels increased in CKD, especially in later stage and in a dialysis patient [*Bramantya R et al., 2020*].

Only a few research have examined the link between NLR and renal function in patients with hypertensive crises. The purpose of this study was to determine whether NLR corresponds with renal function in individuals experiencing an acute hypertensive crisis.

METHODS

Patients and Study Design

The study took place at Citra Husada Hospital in Jember, East Java, Indonesia. The data for this cross-sectional study were gathered from medical records between January and February 2020. We included 167 consecutive patients who were admitted to the hospital between January and December 2019 with the diagnosis of hypertensive crisis. Incomplete information from medical records was a criterion for exclusion. The study was approved by the Ethics Committee of Citra Husada Hospital, Jember, Indonesia (approval no.158/RSCH/I/2020).

Clinical and Laboratory Assessment

Demographic information (age and sex) and history of hypertension were recorded for each patient on admission. The BP of patients was measured on the presentation at the emergency room with an automated device (Omron) or a manual device (Riester). HE was defined as a severe elevation of SBP >179 *mmHg* or DBP >109 *mmHg* with target organ damage. HU was defined as a marked rise in blood pressure in the absence of evidence of target organ damage.

Blood samples were obtained from each patient at admission in the emergency room for complete blood count and serum creatinine (SCrz). The NLR was counted as neutrophil count (*/μL*) divided by lymphocyte count (*/μL*). The eGFR rate (eGFR, *ml/min*/1.73 m^2) was calculated with the Modification of Diet in Renal Disease equation [*Levey A et al., 1999*] eGFR = 186 x [SCr x 0.0011312] $^{-1.154}$x [age (years)] $^{-0.203}$x [0.742 if female] x [1.212 if black] where GFR (*ml/min*/1.73 m^2), Scr (μmol/L) is serum creatinine, age (years).

Statistical Analysis

IBM SPSS, version 24.0, was used to analyze the data. If the distribution was not normal, continuous data were presented as mean standard deviation (e.g., age) and median (min.-max). Categorical data are expressed as number (%). The independent t-test was used to compare two continuous variables with a normal distribution and the Mann-Whitney U test was used to compare two

TABLE 1.

Baseline Characteristics

Variable	n = 167
Age (year), mean ± SD	58.34 ± 11.56
Sex, n (%)	
Male	68 (40.7)
Female	99 (59.3)
History of Hypertension, n (%)	85 (50.9)
Systolic blood pressure *(mmHg)*, median (Min.-Max.)	190 (132-260)
Diastolic blood pressure *(mmHg)*, median (Min.-Max.)	110 (80-190)
Hypertensive Crisis, n (%)	
Hypertensive urgencie	13 (7.8)
Hypertensive emergencies	154 (92.2)
Target Organ Damage, n (%)	
Cardiac	38 (22.8)
Pulmonary Edema	6 (3.6)
Myocardial Infarction	7 (4.2)
Congestive Heart Failure	25 (15.0)
Cerebral Event	107 (64.1)
Stroke Hemorrhagic	22 (13.2)
Stroke Non-Hemorrhagic	84 (50.3)
Transient Ischemic Attack	1 (0.6)
Renal Failure	11 (6.6)
Neutrophil (*/μL*), median (Min.-Max.)	74 (47-94)
Lymphocyte (*/μL*), median (Min.-Max.)	18 (3-40)
neutrophil-to-lymphocyte ratio, median (Min.-Max.)	4.33 (1.18-31.33)
serum creatininer (*mg/dL*), median (Min.-Max.)	1.30 (1.00-21.00)
estimated glomerular function rate (*ml/min/1.73m²*), median (Min.-Max.)	51.70 (2.5-112.9)

continuous variables with an abnormal distribution. When comparing more than two continuous variables with normal distribution, one-way analysis of variance (ANOVA) was used; when the data were not normally distributed, the Kruskal-Wallis test was used. Chi-square test was applied for the comparison of the categorical variable. Spearman's rho test was performed to analyze the associations between NLR and eGFR. A *p*-value below 0.05 indicated a significant difference.

Results

A total of 167 patients were analyzed, 3 patients were excluded due to incomplete data in medical records. Baseline characteristics are shown in Table 1. Hypertensive crisis proportion was lower in male (40.7%, 68 patients) than females (59.3%, 99 patients). The HE incidence was higher (92.2%, 154 patients) with cerebral events as the most frequent target organ damage (64.1%, 107 patients).

Clinical characteristics of hypertensive emergencies and hypertensive urgencies of patients are shown in Table 2. Lymphocyte count and NLR were significantly lower in HE patients (p=0.029 and p=0.033, respectively), while eGFR was significantly higher in HE patients (p=0.046). There were no significant differences in age, sex, history of hypertension, BP, neutrophil count, and SCr between HU and HE patients.

Table 3 shows clinical characteristics of target organ damage in HE. SBP was significantly different in cerebral event, cardiac, and renal groups (p=0.023), with the highest median of SBP in the cerebral event 193 (154-260), followed by cardiac 190 (132-230) and renal 190 (150-213). In contrast, DBP was not significantly different. SCr and eGFR were also significantly different (p=0.000 and p=0.000, respectively). The highest median of SCr was 6.90 (1.00-21.00) followed by the lowest median of eGFR 7.40 (2.50-53.70) in the renal group.

Correlations were calculated between NLR and eGFR in HU and HE patients with analysis in each group of target organ damage (Table 4). Significance correlation was found in the HE and cardiac event groups (p=0.028 and p=0.030, respectively). The correlation between NLR and eGFR in HE patients was negative, with a very weak score (r=-

Table 2.

Clinical Characteristics of Hypertensive Emergencies and Hypertensive Urgencies

Variable	Hypertensive urgencies	Hypertensive emergencies	p
Age (year), mean ± SD	55.54 ± 12.91	58.57 ± 11.45	0.488[a]
Sex			
Male	3 (23.1%)	65 (42.2%)	0.244[b]
Female	10 (76.9%)	89 (57.8%)	
History of Hypertension			
Yes	4 (30.8%)	81 (52.6%)	0.156[b]
No	9 (69.2%)	73 (47.4%)	
Systolic blood pressure (*mmHg*), median (min.-max.)	190 (156-215)	191 (132-260)	0.577[c]
diastolic blood pressure (*mmHg*), median (min.-max.)	114 (90-180)	110 (80-190)	0.511[c]
Neutrophil (*/μL*), median (min.-max.)	70 (49-84)	75 (47-94)	0.072[c]
Lymphocyte (*/μL*), median (min.-max.)	24 (11-40)	17 (3-40)	0.029[c]*
Neutrophil-to-lymphocyte ratio, median (min.-max.)	2.92 (1.23-7.64)	4.40 (1.18-31.33)	0.033[c]*
Serum creatinin (*mg/dL*), median (min.-max.)	1.60 (1-3)	1.20 (1.00-21.00)	0.083[c]
Estimated glomerular function rate (*ml/min/1.73 m²*), median (min.-max.)	43.00 (20.90-78.00)	53.55 (2.50-112.90)	0.046[c]*

Notes: *[a]-Independent-t-test: p<0.05; significant, [b]-Chi-square test: p<0.05; significant. [c]-Mann-Whitney U test: p<0.05; significant*

TABLE 3.

Clinical Characteristics of Target Organ Damage in hypertensive emergencies

Variable	Cerebral Event n=107	Cardiac n=38	Renal n=11	p
Age (year), mean ± SD	59.35 ± 10.93	58.21 ± 12.22	55.09 ± 14.36	0.499[a]
Sex				
Male	49 (45.8%)	13 (34.2%)	3 (27.3%)	0.278[b]
Female	58 (54.2%)	25 (65.8%)	8 (72.7%)	
History of Hypertension				
Yes	60 (56.1%)	19 (50.0%)	4 (36.4%)	0.414[b]
No	47 (43.9%)	19 (50.0%)	7 (63.6%)	
Systolic blood pressure (*mmHg*), median (min.-max.)	193 (154-260)	190 (132-230)	190 (150-213)	0.023[c]*
Diastolic blood pressure (*mmHg*), median (min.-max.)	111 (80-190)	110 (90-144)	110 (87-126)	0.161[c]
Neutrophil (*/μL*), median (min.-max.)	75 (53-94)	73 (47-90)	82 (60-93)	0.065[c]
Lymphocyte (*/μL*), median (min.-max.)	17 (3-39)	19 (3-40)	14 (4-31)	0.076[c]
Neutrophil-to-lymphocyte ratio, median (min.-max.)	4.56 (1.36-31.33)	3.84 (1.18-27.00)	5.86 (1.94-23.25)	0.080[c]
Serum creatinine (*mg/dL*), median (min.-max.)	1.20 (1.00-17.00)	1.30 (1.00-5.00)	6.90 (1.00-21.00)	0.000[c]*
Estimated glomerular function rate (*ml/min/1.73 m²*), median (min.-max.)	54.1 (3.0-107.1)	54.0 (14.2-112.9)	7.40 (2.50-53.70)	0.000[c]*

NOTES: *[a]-One-Way ANOVA: p<0.05; significant; [b]-Chi-square test: p<0.05; significant; [c]-Kruskal-Wallis test: p<0.05; significant*

TABLE 4.

Correlation between NLR and eGFR in HU and HE patients

Variable	Neutrophil-to-lymphocyte ratio Med (min.-max.)	Estimated glomerular function rate Med (min.-max.)	p	r	r^2
Hypertensive urgencies	2.92 (1.23-7.64)	43.00 (20.90-78.00)	0.109	-0.465	0.216
Hypertensive emergencies	4.40 (1.18-31.33)	53.55 (2.50-112.90)	0.028*	-0.177	0.208
Cerebral Event	4.56 (1.36-31.33)	54.10 (3.00-107.10)	0.422	-0.078	0.006
Cardiac	3.84 (1.18-27.00)	54.00 (14.2-112.9)	0.030*	-0.357	0.127
Renal	5.86 (1.94-23.25)	7.40 (2.50-53.70)	0.629	0.164	0.190

NOTE: **Spearman's rho test: p<0.05; significant*

0.177). The coefficient of determination 0.208 means that each increase in NLR will be followed by a decrease of eGFR 20.8% in HE patients. The correlation between NLR and eGFR in HE patients with cardiac as the target organ damage was also negative with a weak score (r=-0.357). The coefficient of determination 0.127 means that each increase in NLR will be followed by a decrease of eGFR 12.7% in HE patients with cardiac as target organ damage.

We also performed NLR and eGFR correlation analysis in all patients. The result was significant (p=0.036), with a very weak score (r=-0.163) and coefficient of determination 0.266. Each increase in NLR was followed by a decrease of eGFR 26.6% (Figure).

DISCUSSION

This study demonstrated that increased NLR had a significant risk of decreased renal function, which was evaluated by eGFR. The result of the analysis was significant, with a very weak score.

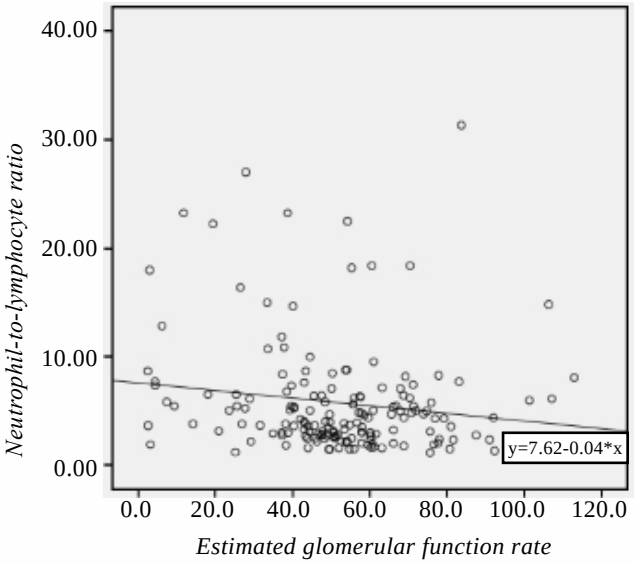

Figure 1. *Correlation between Neutrophil-to-lymphocyte ratio and Estimated glomerular function rate*

In this study, each increase in NLR will be followed by a decrease in eGFR. SCr and eGFR were also significantly different. This study also shows a significant correlation between the HE groups and the cardiac event group. The correlation between NLR and eGFR in HE patients was negative, with a very weak score. These results are also similar in HE patients with cardiac as the target organ damage.

This study shows that increasing in NLR will be followed by a decrease of eGFR in HE patients with cardiac as target organ damage. Increased NLR levels were found to be a significant predictor of adverse renal outcomes in one investigation. It was caused by increasing oxidative stress or inflammatory markers [*Yoshitomi R et al., 2019*]. NLR could be a valuable marker to predict renal dysfunction, cardiovascular complication, and stress response of the patients. This ratio is composed of two different pathways of immunity. The neutrophil count shows the inflammation process [*Yuan Q et al., 2019*]. Lymphocyte count shows general health and physiological stress [*Sevencan N, Ozkan A, 2018*]. In a four-year follow-up research, NLR was identified as an independent predictor of the development of significant adverse cardiac events in the diabetic group [*Azab B et al., 2012*]. This parameter is inexpensive to collect, generally available, and somewhat stable when compared to other parameters (ex: leukocyte, neutrophil, lymphocyte, monocyte, and platelet counts) [*Akase T et al., 2020*].

In this study, hypertensive crisis proportion is lower in males than females. The HE incidence is higher in patients with cerebral events as the most frequent target organ damage. SBP is significantly different in the cerebral event, cardiac, and renal groups, with the highest median of SBP in the cerebral event 193 (154-260), followed by cardiac 190 (132-230) and renal 190 (150-213). In contrast, DBP was not significantly different. In HE patients, lymphocyte count and NLR were much lower, although eGFR was significantly greater. Between HU and HE patients, there were no significant differences in age, sex, history of hypertension, blood pressure, neutrophil count, or SCr.

This study had some limitations. The selection of subjects was limited only to one hospital. The data were collected from medical records, so the information was based on the previous examiners, and the baseline NLR and eGFR were not known due to the cross-sectional study design. Cohort studies need to be done to decrease the bias of information and standardize data collection procedures.

Conclusion

This study suggested a relationship between the increasing NLR with decreasing eGFR in hypertensive crisis patients. Further studies are needed to evaluate whether the increase in NLR was an acute or chronic disease process.

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BASELINE CHARACTERISTICS, CLINICAL PROFILE AND OUTCOMES OF PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA A SINGLE CENTER EXPERIENCE IN SOUTH INDIA

Karanth S.[1], Karanth S.[2], Rao R.[1*]

[1] Department of Medicine, Kasturba Medical College, Manipal Academy of Higher Education, Manipal, India
[2] Department of Hematology, A. J Hospital and Research Center, Mangalore, India

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Abstract

Paroxysmal nocturnal hemoglobinuria is a rare, nonmalignant, acquired disease that manifests as hemolytic anemia, bone marrow failure and thrombosis that occurs due to a somatic mutation in the PIG-A gene. Here, regardless of treatment, we assessed the baseline characteristics and clinical profile of patients with a confirmed paroxysmal nocturnal hemoglobinuria diagnosis or detectable paroxysmal nocturnal hemoglobinuria clone.

A total of 16 patients, 10 male and 6 females, who presented to our center between April 2016 to March 2020 and diagnosed with paroxysmal nocturnal hemoglobinuria were provided treatment for the management of their condition. Information regarding patients' medical and treatment history, comorbid conditions, paroxysmal nocturnal hemoglobinuria clone size, disease characteristics and outcomes, symptoms, paroxysmal nocturnal hemoglobinuria -specific treatments, paroxysmal nocturnal hemoglobinuria -related events, morbidity, mortality, and quality of life were collected. Data including hemoglobin levels, transfusion requirements, renal dysfunction, thrombotic events and other laboratory data were collected.

Frequently reported symptoms included fatigue (75%), dyspnea (44%), hemoglobinuria (25%), and abdominal pain (44%) with an overall 44% hospitalization rate due to related complications. Median granulocyte PNH clone size was 36.3% (range 14-78 percent). Overall, 37.5% patients had classical paroxysmal nocturnal hemoglobinuria and 62.5% patients had paroxysmal nocturnal hemoglobinuria with secondary bone marrow disorders. Post individualized therapy; the mortality rate was 18.75%. Presently 13 patients are on follow up with either steroids (38.5%), steroids with danazol (23%), cyclosporine (7.7%), thalidomide (7.7%), or intermittent blood product support (23%).

The data from this study can be used to identify the patterns that would indicate the necessity for a diagnosis of paroxysmal nocturnal hemoglobinuria and to identify the diagnostic outcomes.

Keywords: *paroxysmal nocturnal hemoglobinuria, thrombosis, hemolysis, management.*

Introduction

Paroxysmal nocturnal hemoglobinuria (PNH) is a rare, acquired bone marrow disorder that is primarily characterized by hemolytic anemia, thrombosis, peripheral blood cytopenias, and in most cases, a poor quality of life [*Brodsky R, 2014; Schrezenmeier H et al., 2014*]. The incidence and prevalence of PNH is estimated to be approximately 1.3 per million annually and 16 per million, respectively. It usually develops between 30 – 40 years of age, although initiation of physical symptoms can occur at any age [*Hill A, 2006*]. Uncontrolled complement activity in PNH results in systemic complications, mostly due to intravascular hemolysis and platelet activation [*Schrezenmeier*

Address for Correspondence:
Raghavendra Rao
Associate Professor, Department of Medicine, Kasturba Medical College, Manipal Academy of Higher Education, Manipal, India
Tel.: +91 9901731924
E-mail: ragsmanipal1983@gmail.com

H et al., 2014]. Paroxysmal nocturnal hemoglobinuria is a life-threatening disease where the cellular abnormalities develop from an acquired mutation in the phosphatidylinositol glycan class A) gene in a self-renewing hematopoietic stem cell, followed by clonal expansion. The first step in the synthesis of the glycosylphosphatidylinositol (GPI) anchor is mediated by the PIGA protein. The GPI anchor is a glycolipid that links various cell surface proteins to the plasma membrane of hematopoietic cells. The mutation results in a deficiency of glycosylphosphatidylinositol (GPI)-anchored complement regulatory proteins, including CD55 and CD59, on the surface of blood cells that causes complement□mediated intravascular hemolysis, promotion of inflammatory mediators, and systemic hemoglobin release [*Parker C et al., 2005; Hill A et al., 2013;Schrezenmeier H et al., 2014;*]. A marked increase in the risk of thromboembolism is observed in patients with chronic hemolysis, that could eventually lead to target organ damage, and ultimately, death [*Parker C et al., 2005*].

Paroxysmal nocturnal hemoglobinuria has been classified into three subcategories, such as classic PNH where the patients have clinical evidence of intravascular hemolysis, but have no evidence of another defined bone marrow abnormality, PNH in the setting of another specified bone marrow disorder where the patients have clinical and laboratory evidence of hemolysis but also have concomitantly, or have had a history of, a defined underlying marrow abnormality (e.g., PNH/aplastic anemia or PNH/refractory anemia- myelodysplastic syndrome), and subclinical PNH (PNH-sc) where the patients have no clinical or laboratory evidence of hemolysis [*Parker C et al., 2005*].

Thrombosis, which occurs in up to 40% of patients, is responsible for the greatest number of deaths in PNH, inspite of its rare symptomatic presentation. Thromboembolism accounts for nearly 40% to 67% of the deaths due to PNH. The frequency of occurrence of one thromboembolic event during the course of this disease is 29% to 44%, although the reasons behind the sudden thrombotic event remain an enigma [*Hill A et al., 2013; Schrezenmeier H et al., 2014*]. The most frequent symptom in patients with PNH is fatigue; the other symptoms include abdominal pain, headache, shortness of breath (dyspnea), dysphagia, and erectile dysfunction. These symptoms can be debilitating and significantly reduce the quality of life (QoL) of patients with PNH. Another risk associated with PNH is chronic renal tubular damage caused by microvascular thrombosis and accumulation of iron deposits.

Although allogeneic bone marrow transplantation is the only potentially curative therapy for PNH, it is offered for refractory PNH, secondary PNH patients with profound cytopenias or malignant transformation. This therapy is association with considerable morbidity and mortality [*Schrezenmeier H et al., 2014*]. Supportive measures such as blood transfusions and anticoagulation therapy have been the favored therapies for the management of PNH; however, prophylactic anticoagulation has been associated with an increased risk of bleeding complications [*Schrezenmeier H et al., 2014*]. Since complement-mediated cytolysis results in hemolysis, PNH therapy should involve inhibition of the complement. The complement inhibitor eculizumab, a humanized monoclonal antibody against complement C5, is a widely effective therapy for patients with classical PNH. Eculizumab has been shown to effectively control the signs and symptoms of hemolysis and markedly improved the quality of life of PNH patients. Notably, significant adverse events with eculizumab have not been reported. Moreover, corticosteroids have reportedly improved hemoglobin levels and reduced hemolysis in some PNH patients.

In the current study, we assessed the baseline characteristics and clinical profile of patients with a confirmed paroxysmal nocturnal hemoglobinuria diagnosis or detectable paroxysmal nocturnal hemoglobinuria clone and initiated individualized treatment based on their symptoms and associated risk factors.

To overcome it
is possible, due to the
uniting the knowledge and
will of all doctors in the world

MATERIALS AND METHODS

Patient Population

The study was conducted between April 2016 to March 2020 at our centre after obtaining permission from Institutional Ethics Committee (IEC: 613/2016). The work was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). Informed consent was obtained from all the participants. A total of 16 patients, 10 males and 6 females, aged 14 – 66 years, were included in the study. Inclusion criteria involved a clinical diagnosis of PNH or detection of ≥5% PNH clone. A paroxysmal nocturnal hemoglobinuria clone was defined as a population of granulocytes and/or erythrocytes deficient in GPI. Informed consent was obtained from all the participants.

Data Collection

Information regarding patients' medical and treatment history, comorbid conditions, paroxysmal nocturnal hemoglobinuria clone size, disease characteristics and outcomes, symptoms, paroxysmal nocturnal hemoglobinuria-specific treatments, paroxysmal nocturnal hemoglobinuria-related events, morbidity (including myeloproliferative/ myelodysplasia/aplastic anemia, other malignancies), mortality, and quality of life were collected and recorded.

Data collected from clinical assessments included hemoglobin levels, transfusion requirements, renal dysfunction, thrombotic events (identified using major adverse vascular event categories), and other laboratory data. Data were presented as frequency (%).

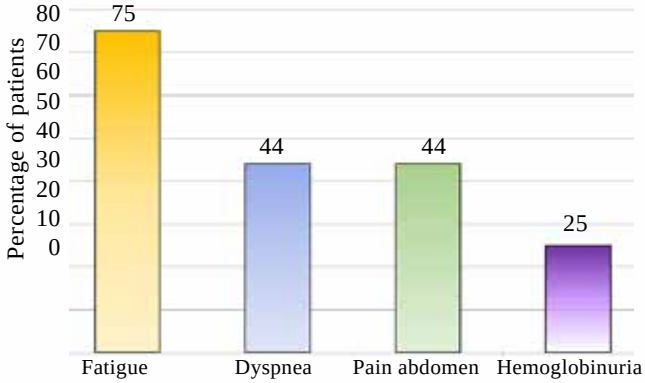

FIGURE 1. Symptoms presented by the patients at their first visit.

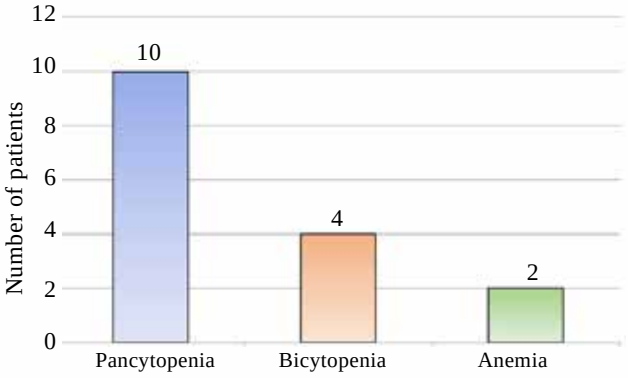

FIGURE 2. Clinical presentations of the patients with paroxysmal nocturnal hemoglobinuria.

Management of Paroxysmal Nocturnal Hemoglobinuria

The therapies for the management of paroxysmal nocturnal hemoglobinuria included anticoagulation, immunosuppression, bone marrow transplant, and intermittent blood product support. The various drugs and their doses are listed in Table 1.

RESULTS

Demographic Characteristics

Here, we report the characteristics of the 16 patients, 10 males and 6 females, diagnosed with paroxysmal nocturnal hemoglobinuria between April 2016 to March 2020 and treated at our centre. The median age of the patients was 39 years. As shown in Figure 1, the patients frequently presented with symptoms, such as fatigue (75%), dyspnea (44%), hemoglobinuria (25%), and abdominal pain (44%). Overall, 44% of the patients had been hospitalized due to paroxysmal nocturnal hemoglobinuria-related complication. Moreover, 6.25% of patients had a history of thrombotic events and 12.5% a history of impaired renal function.

Clinical Diagnosis

Blood analysis at first visit revealed that 37.5% patients had classical paroxysmal nocturnal hemoglobinuria and 62.5% patients had paroxysmal nocturnal hemoglobinuria with secondary bone marrow disorders (BMDs). The patients with secondary bone marrow disorders were diagnosed either with aplastic anemia or hypoplastic anemia (43.75%) and myelodysplastic syndromes (18.75%). As shown in Figure 2, 62.5% of the patients had pancytopenia, 25% had bicytopenia, and 12.5% had anemia.

Flow cytometric analysis of the peripheral blood revealed that the median granulocyte parox-

ysmal nocturnal hemoglobinuria clone size was 36.3% (range 14%-78%). Patients without a history of BMD presented with a larger clone size, the median clone size being significantly larger compared to patients who had at any point been diagnosed with associated BMD (43.3% vs. 32.1%).

Efficacy of the Therapies for Paroxysmal Nocturnal Hemoglobinuria

Table 2 lists the therapies provided to the patients based on their clinical diagnosis. Anticoagulation therapy was provided to (6.25% of the patients, immunosuppressive therapy to 66.25% patients, bone marrow transplant to 18.75% patients, and intermittent blood product support to 18.75% of the patients. However, unfortunately three patients died even after the therapy. Presently, the remaining 13 patients are on follow up therapy either with steroids (38.5%;5/13), steroids along with danazol (23%;3/13), cyclosporine (7.7%;1/13), thalidomide (7.7%;1/13), and intermittent blood product support (23%; 3/13).

Discusion

The current clinical investigation evaluated the base-line characteristics of the patients who reported to our centre with symptoms of paroxysmal nocturnal hemoglobinuria. Our patient population had a high morbidity rate at enrollment; 43.75% of patients had aplastic anemia or hypoplastic anemia, 18.75% had myelodysplastic syndromes, 12.5% had a history of impaired renal function, and 6.25% of the patients had experienced at least one thrombotic event. Three patients died inspite of therapy while in the remaining 13 patients; therapeutic support was continued either with immunosuppressive drugs or blood transfusion. Paroxysmal nocturnal hemoglobinuria occurs in both men and women equally. Although it is usually diagnosed in young adulthood, it can occur at any age. The symptoms could be many or just a few, the most frequently reported symptom being fatigue. The severity of the condition depends on the number of faulty blood cells in the patients' body. In our study, therapy was individualized depending on the severity of the patients' condition and the presence of other risk factors.

At present, allogeneic bone marrow transplantation is the only curative therapy for the eradication of paroxysmal nocturnal hemoglobinuria clone in patients with classical PNH and aplastic anemia /PNH. However, it is less popular due to its association with considerable morbidity and mortality. Till date, a substantially small proportion of patients have availed this therapy [Brodsky R, 2010]. Importantly, Human leukocyte antigen (HLA)-identical sibling bone marrow transplantation is an effective therapeutic option for PNH, which is effective even in the hemolytic phase of the disease. Bone marrow transplant is recommended when patients have significant risk factors, such as thrombosis, pancytopenia, myelodysplastic syndromes or acute leukemia, thrombocytopenia, or a marked need for transfusions [*Raiola A et al., 2000*]. Three of the sixteen patients only qualified for bone marrow transplant.

Supportive measures, such as anticoagulation therapy and blood transfusions are the oldest management therapies for PNH; however, anticoagulation therapy has been reported to increase the risk of thromboembolism in patients with PNH, and is associated with an increased risk of bleeding complications. Therefore, anticoagulation therapy is justified for PNH patients with thrombotic episodes who are not associated with contraindications like severe thrombocytopenia. Low molecular weight heparin has been recommended for cases without contraindication to full anticoagulation [*Brodsky R, 2009*]. It has been reported that primary prophylaxis with warfarin prevents thrombosis with acceptable risks in PNH patients. Anticoagulation therapy may thus improve survival and reduce mortality in PNH patients [*Hall C et al., 2003*]. Here, we used enoxaparin injection (1 *mg/kg*) for anticoagulation therapy, which was administered only to one patient. Treatment of anemia, the most common PNH problem is best done by blood transfusions. In our study, blood transfusions were performed in 3 patients. In a blood transfusion, whole blood, or parts of blood from a donor are put right into the patients' bloodstream. Two types of transfusions are available for PNH patients; they are red blood cell transfusion and platelet transfusion [*Schrezenmeier H et al., 2010*]. Blood transfusions have been also indicated in life-threatening cases like acute paroxysm. Transfusion of red blood cell is essential both in the steady state and at the time of chronic hemolytic anemia aggravation. However, the blood transfu-

sion regime is best tailored to the individual patient taking into account the rate of fall in hemoglobin since the last count, the objective clinical assessment, and the subjective state of the patient, also in relationship to physical exertion [*Olutogun T et al., 2015*].

Another method used for the treatment of PNH is the immunosuppressive therapy, which was used in most of the patients during the study as well as in the follow-up period. It lowers the immune response of the patient and prevents it from attacking the bone marrow. It is mostly appropriate for PNH patients with aplastic anemia. A standard approach for treating acquired aplastic anemia includes immunosuppressive therapy comprising of anti-thymocyte globulin along with cyclosporine [*Sugimori C et al., 2015*]. Although the remission rate in patients is significantly high, it might become harmful in cases with an increased risk of opportunistic infections, particularly in the absence of any remission and also if aplastic anemia is associated with significant PNH clone, then use of ATG is not appropriate from treatment perspective [*Sugimori C et al., 2015*]. Corticosteroids, such as prednisolone, either alone or in combination with an androgen like danazol have also been used for the treatment of PNH. Despite a paucity of randomized trials affirming the effectiveness of oral steroids in hemolysis reduction in PNH, they have been used for the management of acute episodes because of their immediate effects. A continued use of high dosages of corticosteroids has reportedly been associated with substantial side effects; therefore, the International PNH Interest Group has recommended pulse doses [*Ghosh K et al., 2013*]. The mode of action of steroids for preventing hemolysis involves the inhibition of complement activation by an alternate pathway or the dampening inflammation that stimulates activation of complement [*Parker C et al., 2005; Ghosh K et al., 2013*]. According to a study by Issaragrisil et al., the administration of prednisolone in patients with a short disease interval increases the chances of treatment response. They recommended the continuation of treatment for at least 3 months before assessing the outcome, although a longer follow-up period is needed to ascertain the toxicity and infectious complications of the therapy [*Issaragrisil S et al., 1987*]. Similar to prednisolone, the mechanism of PNH amelioration by danazol is also through complement inhibition by resisting osmotic lysis. Another immunosuppressant used in our study was thalidomide, which mediates its biological effects through its immunomodulatory, anti-inflammatory, and anti-angiogenic properties [*Strupp C et al., 2002*]. It has been shown to decrease the T-helper (CD4): T-suppressor (CD8) cells ratio [*Hassan I et al., 2015*].

Currently, novel complement inhibitors are being developed. The assessment of thrombotic risk is of paramount importance; hence, identification of new markers for thrombotic risk is warranted to achieve a better risk-based prophylactic antithrombotic management [*Devos T et al., 2018*].

CONCLUSION

This study was undertaken to investigate the baseline characteristics, clinical behavior, and outcome in patients with paroxysmal nocturnal hemoglobinuria. Since all treatment modalities have their positive outcomes and adverse effects, individualization of therapy based on the discretion of the treating personnel and the clinical presentations of the patient is the best approach for the treatment of PNH. The data from this study can be used to identify the patterns that would indicate the necessity for a diagnosis of PNH and to identify the diagnostic outcomes. Information about PNH is insufficient, and this study could be useful in the diagnosis of other patients with PNH and ultimately improve their medical outcomes.

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TYPE II DIABETES MELLITUS AS AN IMPORTANT RISK FACTOR OF CANCER OF PANCREAS: FINDINGS OF NARRATIVE REVIEW

ALDUKHI S.A.

Department of Family Medicine, King Saud University Medical City, Riyadh, Saudi Arabia

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ABSTRACT

Diabetes mellitus is one of the foremost concerns in high as well as low-middle income nations. Around 45% of cases of pancreatic carcinoma are expected to appear as incident cases of diabetes mellitus; nevertheless, the type of relationship between type two diabetes mellitus and pancreatic carcinoma is however controversial. Therefore, we carried out a narrative review to synthesize the findings of the relationship between diabetes mellitus and cancer of the pancreas.

We approached Google Scholar and PubMed to carry out the searches for relevant articles. We undertook a narrative review of eligible research articles that were available in the English language in developed and developing countries from 2015 to 2020. All primary research articles were examined for information pertinent to the objective. All references of the eligible articles were also reviewed to make sure to include all relevant articles in the review.

The included studies explored the association between diabetes mellitus and malignancy of the pancreas. We found the results of the review to be supportive of our hypothesis. Numerous biologic pathways and processes have been highlighted as a shred of evidence that alludes to the association between diabetes mellitus and malignancy of the pancreas. Abnormalities in metabolism, immunologic pathways, and hormonal features of diabetes mellitus may increase the risk of cancer development.

Further, studies have shown that increased production of insulin and insulin-like growth factors may increase the probability of malignancy of the pancreas by upregulating cellular proliferation and thereby abnormal cancerous cells.

There is evidence that shows that diabetes mellitus increases the risk of several cancers such as malignancy of the pancreas. On the basis of the evidence from this review, it is recommended for clinicians to screen people for diabetes mellitus and also monitor the existing patients with diabetes mellitus to screen for pancreatic cancer in a timely manner.

KEYWORDS: *diabetes mellitus, pancreatic cancer*

INTRODUCTION

Cancer of the pancreas is found to be the most prevalent malignancy across the globe and a five-year survival rate is about 7%, and it has been found that for the majority of patients, mortality ensues in six months following diagnosis [*Ryan D, Hong T, 2014; Mizrahi J et al., 2020*]. Cancer of the pancreas holds a miserable prognosis due to a five-year survival rate of < 5%, and most of the patients die within six months after they are diagnosed with malignancy [*Hezel A et al., 2006*]. The incidence and rate of mortality of this cancer are about 7.5% and 7.0%, respectively after adjusting for age. Furthermore, the majority of the patients are not considered eligible for the surgery due to the locally advanced spread of cancer in the body [*Kuuselo R et al., 2007*].

It is imperative to identify high-risk individuals to increase survival and reduce the morbidity and mortality associated with the cancer of the pancreas. In order to do this, causes and risk factors of pancreatic cancer would need to be identified as its etiology remains uncertain except few demographic and behavioral reasons such as age, smoking status, enhanced body weight, and positive

ADDRESS FOR CORRESPONDENCE:
Sarah Abdullah Aldukhi
Department of Family Medicine
King Saud University Medical City
P.O Box 14115, Riyadh 4545, Saudi Arabia
Tel.: 00966 50 542 4888
E-mail: Sara.Abdullah2500@hotmail.com

family history of pancreatic cancer [*Wiseman M, 2008*]. For example, around one-quarter to one-third of pancreatic cancer is explained by smoking while less than 10% of the diagnosed cases are reported among <50 years old individuals [*Lowenfels A, Maisonneuve P, 2005*]. In addition to this, compelling data have suggested that Diabetes mellitus (DM) increases the risk of numerous malignancies, such as malignancy of the pancreas [*Giovannucci E et al., 2010*], and DM can increase the stage of cancer and death from cancer [*Griffiths R et al., 2012; Toriola A et al., 2014*]; however, the results are found to be conflicting. Additional, it is not still established whether DM is a disposing risk factor or a possible result of tumor growth, or both [*Gapstur S et al., 2000*].

Around 45% of cases of pancreatic carcinoma are expected to appear as incident cases of DM; nevertheless, the type of relationship between type two DM and pancreatic carcinoma is, however, contentious [*Ben Q et al., 1990*]. For example, there is a debate about whether DM leads to malignancy of the pancreas or is the result of malignancy of pancreas and the existing evidence from individual observational studies considers DM as a potential cause for malignancy of pancreas [*Esposito K et al., 2012*]. However, the evidence from such studies needs to be synthesized after reviewing the findings critically. Therefore, we carried out a narrative review to synthesize the findings of the association between DM and malignancy of pancreas. The worse prognosis of pancreatic cancer and the absence of effective treatment for a long time make it worth studying the pathogenesis of cancer and its association with the DM.

Materials and methods

We approached Google Scholar and PubMed to carry out the searches for relevant articles. We undertook a narrative review of eligible research articles that were available in the English language in developed and developing countries from 2015 to 2020. All primary research articles were examined for information pertinent to the objective. All research studies in the above-mentioned databases were searched using search terms such as “association between DM and Pancreatic cancer”, “relationship between DM and malignancy of pancreas”, “DM as a risk factor for malignancy of pancreas”, “DM and malignancy of pancreas”, “Type 2 DM leads to malignancy of pancreas”, “Type 2 DM and malignancy of pancreas”.

We included studies from high and low-middle-income countries and assessed the full-text articles of the eligible studies.

All references of the eligible articles were also reviewed to make sure to include all relevant articles in the review. After searching for different databases and following the above criteria, finally, studies having full-text articles were assessed and were made part of the review.

Results of the Narrative review

Mechanism of action for the relationship between DM and malignancy of pancreas

Type 2 DM is a metabolic disorder with a characteristic increased level of blood glucose levels that happens as a result of malfunctioning insulin excretion by islets of pancreas while responding to increased blood glucose levels after the meal as well as insulin resistance to insulin at the periphery and production of glucose in a dysregulated manner [*Unger R, Orci L, 2010*]. There is a relationship between DM and obesity that has risen over time worldwide. Among obese individuals, the insulin response further deteriorates at the cellular level, which means there is increased resistance of tissues to insulin, increases the burden on the pancreas to produce increased amounts of insulin [*Unger R, Orci L, 2010*]. This in turn will result in an increased amount of insulin in the blood, which is further triggered by genes and environmental factors [*Stumvoll M et al., 2005*].

Insulin works to regulate the levels of glucose in the blood and also regulate the metabolism of lipids and by acting as a possible growth factor in the multiplication of cells and increases in the formation of blood vessels. Though DM is likely to rising the probability of various forms of cancers, the threat to cause malignancy of the pancreas has been found to be very high

To overcome it is possible, due to the uniting the knowledge and will of all doctors in the world

[*Burney S et al., 2014*]. Cancerous alteration of pancreatic tissues with more changes in the epithelial cells of ducts of exocrine part of the pancreas, when allied with raised production of insulin impending from the endocrine part of the pancreas, nurture quickly and perhaps such a relationship might be the reason for the distressing consequence of cancer of the pancreas [*Fokas E et al., 2015*].

Results of studies supporting our hypothesis

The included studies explored the association between DM and malignancy of the pancreas. The findings of the review were supportive of our hypothesis [*Li D et al., 2015*]. For example, a study conducted by Donghui Li and co-authors found that newly diagnosed DM is a substantial impartial determinant for the hazard of death in patients with malignancy of the pancreas (p=0.002). Authors found that DM overall and newly diagnosed DM, in particular, is related to adverse consequences for pancreatic cancer [*Li D et al., 2015*]. Likewise, another observational study was performed by Yun Xia and colleagues who identified cases of malignancy of the pancreas from some database in the UK and identified the controls randomly independent of DM [*Lu Y et al., 2015*]. This was followed by matching factors such as calendar time, age, and sex. Authors found a higher likelihood of pancreatic cancer in patients with Type 2 DM with an Odds ratio of more than 2 [*Lu Y et al., 2015*]. These results indicated that Odds of malignancy of pancreas among patients with DM were two times the Odds of malignancy of pancreas among patients without DM. Authors further reports that patients who were on insulin were 25.57 times likely to develop the pancreatic cancer with imprecise 95% confidence intervals. However, patients on medications such as sulphonylureas were 2.22 times likely to develop the cancer and those who reported using metformin were 1.46 times likely to develop pancreatic cancer when compared to those who were on no medications. According to authors, newly diagnosed DM appears to be an unbiased risk factor for cancer of pancreas. The association between medications for DM and malignancy of pancreas seem to differ in magnitude and precision with higher probability of malignancy of pancreas among insulin users [*Lu Y et al., 2015*].

Another observational study was conducted by using the National Diabetes Register of Sweden to assess the association between DM and the probability of pancreatic cancer [*Sadr-Azodi O et al., 2015*]. They identified 10 controls randomly for each case of the pancreas and matched them with cases on age, sex, and other characteristics related to DM with a ratio of 1:10 cases to controls between 2005 to 2011 [*Sadr-Azodi O et al., 2015*]. According to the authors, diabetic patients with increased concentrations of HBA1c were at higher risk of acquiring malignancy of pancreas with an Odds ratio of about 2 and significant results (p-value: <0.05). The authors also highlighted that there is a lead time of numerous to detect pancreatic cancer among diabetic patients in a timely manner [*Sadr-Azodi O et al., 2015)*].

Another study was conducted in China using data from four cancer registries in four different hospitals [*Zheng Z et al.,2016*]. The authors selected controls by including family members of non-pancreatic cancer patients and conducted interviews with them to explore the correlation between DM and the probability of malignancy of the pancreas. Authors found that apart from smoking, obesity, family history, DM appeared to be the strongest risk factor for malignancy of the pancreas and recommended designing appropriate interventions to prevent pancreatic cancer [*Zheng Z et al.,2016*]. Another study was conducted to find the independent effect of DM on the malignancy of the pancreas on 817 cases and 1756 controls [*Antwi S et al., 2016*]. The study findings revealed three times more danger of malignancy of the pancreas among patients with DM than non-diabetic patients in addition to other risk factors including pro-inflammatory diet and smoking. This was followed by another prospective study on about 512,000 individuals from various areas of China [*Pang Y et al., 2017*]. This large epidemiological study found about 2-fold increase in the risk of pancreatic cancer among diabetic patients with statistically significant results. The risk of cancer was also substantially greater in diabetic patients who were diagnosed with longer duration of DM and patients with previous diagnosis of DM had about 52% excess risk of malignancy of pancreas [*Pang Y et al., 2017*]. Similarly, Setiawan V.W. and co-authors (2019) conducted a research study in 2018 on about 50,000 African American and Latinos in the large cohort. The study found gretaer

than 2-fold increase probability of malignancy of pancreas among diabetic patients, thereby further endorsing the findings of previously conducted observational studies [*Setiawan V et al., 2019*].

Conclusion

Diabetes mellitus is considered an important concern in both high and low-middle income areas. There is a piece of evidence that shows that DM increases the risk of several cancers including pancreatic cancer. Numerous biologic pathways and processes have been highlighted as a shred of evidence that alludes to the correlation between DM and malignancy of the pancreas. Abnormalities in metabolism, immunologic pathways, and hormonal features of DM may increase the risk of cancer development. Further, studies have shown that increased production of insulin and insulin-like growth factors may increase the probability of malignancy of the pancreas by upregulating cellular proliferation and thereby abnormal cancerous cells. Based on the evidence from this review, it is recommended for clinicians to screen people for DM and also monitor the existing patients with DM to screen for pancreatic cancer in a timely manner.

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Yerevan State Medical
University after M. Heratsi.

Address for correspondence:

Yerevan State Medical University
2 Koryun Street, Yerevan 0025,
Republic of Armenia

Phones:

(+37410) 582532 YSMU
(+37410) 580840 Editor-in-Chief
Fax: (+37410) 582532
E-mail: namj.ysmu@gmail.com, ysmiu@mail.ru
URL: http//www.ysmu.am

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Copy editor: Tatevik R. Movsisyan

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Director: A. Muradyan
Armenia, 0002, Yerevan,
Saryan St., 4 Building, Area 2
Phone: (+374 10) 52 02 17,
E-mail: collageltd@gmail.com

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