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A Clinical Study of the Distribution and Morphology of Harris Lines

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ABSTRACT

Harris lines are commonly used in bioarcheology to infer lifestyle in ancient populations; however, their etiology and identification parameters are still under debate. The aim of this study is to observe the distribution of the lesions in a contemporary clinical sample to address their association with etiological factors, age, biological sex, and biogeographical origin. The sample consisted of 561 living individuals from Italy ($n=268$) and Sri Lanka ($n=293$). Radiographic images of long bones (humerus, radius, ulna, femur, tibia, fibula), and information on biological sex and age were collected. Statistical analysis was conducted using Fisher's test, chi-square tests, and odds ratios. Harris lines were significantly more frequent in bones of the lower limb ($p<0.001$), and among Sri Lankan (32%) rather than Italian-grown individuals (20%) ($p=0.001$). No statistically significant differences between sexes were observed ($p=0.790$). No linear trend was found in the distribution of the lesions among age groups; however, their presence was attested among the oldest individuals (>80 years), challenging assumptions about bone remodeling and the evanescence of Harris lines. Additionally, morphological variation in radiopaque lesions was documented. The equal distribution between sexes is coherent with the characteristics and the contextual information of the samples, where no sex-based disparities are reported. This sheds new light on the impact of female biological buffering, as well as enforcing the interpretative role of Harris lines in detecting inequalities between females and males. The differences observed among the two geographical subgroups may be related to the socioeconomic context of growth, highlighting the capacity of Harris lines to reflect variations in access to resources.

1 | Introduction

The understanding of the lifestyle of past populations can be enriched through a bioarcheological approach, particularly by examining bone and dental lesions related to nutritional and pathological stress. Among these lesions are Harris lines (HL), defined as transverse radiopaque bands of increased bone density (typically 5–10%), commonly found on the diaphysis and metaphysis of long bones (Figure [only online]) (Alfonso 2011; Georgiadis and Gannon 2022; Harris 1931; Hummert and Van Gerven 1985; Kulus and Dąbrowski 2019; Suter et al. 2008). HLs can be visualized through radiography, a cost-effective and non-destructive imaging (Elliott 2022), as well as through computed tomography (CT), magnetic resonance imaging, and histological thin sections (Kulus and Dąbrowski 2019). The first description of HLs was provided by Wegner in 1874,

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with subsequent observations in humans by Ludloff (1903) and Harris and Park (1927) (Georgiadis and Gannon 2022). In the field of bioarcheology, HLs not only offer insights into an individual's lifestyle but also provide indications of maternal health during the final stages of gestation following the mother-infant nexus theory (Edwards 1993), as these lesions can manifest in fetuses.

1.1 | Etiology

The etiology of HLs can be described as multifactorial, reflecting not a singular condition but rather resulting from the combination of various environmental, pathological, and physiological processes (Alfonso et al. 2005; Boucherie et al. 2017; Fiscella et al. 2008; Kulus and Dąbrowski 2019). Environmental factors such as malnutrition (including nutritional deficiency, marasma, kwashiorkor), traumatic injuries (Salter–Harris fractures), characteristics of birth, breastfeeding and weaning practices, ethanol consumption, bisphosphonate use, birth order, and birth weight are factors that have been identified as influential (Guzman 1972; Piercecchi-Marti et al. 2006). These factors may act synergistically with one another and be aggravated by other pathological conditions (Beom et al. 2014). Maternal health status and habits during gestation can influence a child's bone growth. In fact, hypertension, hazardous substance intake (including medications and alcohol), infections (e.g., urinary tract infections, tuberculosis), anemia, gestational diabetes, and birth characteristics may potentially contribute to the development of HLs in children (Caffey 1939; Edwards 1993; Reed 1969). Infections occurring during infancy and adolescence have been associated with HL formation (Gindhart 1969; Miller and Rubell 1934), although not necessarily in a one-to-one correspondence (Allison et al. 1974; Ferrozzi et al. 1990). HLs have also been related to metabolic disorders such as scurvy, rickets, vitamin A deficiency, vitamin K deficiency, and food poisoning (mainly associated with fluoride and metals) (Georgiadis and Gannon 2022). Nutritional deficiencies not only reflect general lifestyle and access to food but also exposure to infectious and gastrointestinal pathologies that can compromise vitamin and mineral absorption (Condon-Paoloni et al. 1977). Furthermore, HLs have also been interpreted as the result of the dynamic physiological growth of the bone tissue (Alfonso Durruty 2002; Alfonso 2011; Alfonso et al. 2005; Chaumoître 2007; Gilde 2013; Papageorgopoulou et al. 2011; Symes 1983). Specifically, these authors argue that the dynamic process of bone maturation, which proceeds in phases of growth suppression (or *stasis*, an interruption in bone matrix secretion) and resumption (or *saltation*), can result in the formation of HLs (Alfonso et al. 2005; Magennis 1990).

1.1.1 | Pathogenesis

Endochondral ossification consists of the replacement of the cartilaginous matrix with bone tissue (Mackie et al. 2008), and its temporary interruption may result in growth alterations and radiopaque lesions (Wongdee et al. 2012). Two prevailing theories exist regarding the formation of HLs (Georgiadis and Gannon 2022; Scott and Hoppa 2015). The first posits that it may be attributed to the interruption of chondrocytes' metabolism while osteoblastic activity remains unaffected (Kulus and Dąbrowski 2019). In contrast, the second hypothesis proposes that HLs represent growth recovery lines (Alfonso 2011; Nowak and Piontek 2002b; Park 1964; Papageorgopoulou et al. 2011; Sajko et al. 2011). According to these pathogeneses, the physiological process of bone growth, which proceeds dynamically, could determine the formation of HLs. However, variations in hormones (e.g., GH, T^3) and growth factors (e.g., IGF-I) that influence endochondral ossification characterize both the physiological maturation of bones and pathological alterations (Robson et al. 2002; Slootweg 1993).

Despite their extensive use in bioarchaeology (Berrocal-Zaragoza and Subirà 2008; Fiscella et al. 2008; Geber 2014; Goodman and Clark 1981; Hughes et al. 1996; Lobdell 1984; McHenry and Schulz 1976; Velasco-Vázquez et al. 1999), the morphology, epidemiology, and etiology of Harris lines are still debated (Alfonso et al. 2005; Condon-Paoloni et al. 1977; Garn and Schwager 1967; Miszkiewicz 2015; Nowak and Piontek 2002a). Analyzing a clinical sample with available ante-mortem data may allow us to clarify the interpretation of this lesion to improve its application and interpretation in bioarchaeological contexts. In this sense, this research was conceived as a pioneering effort into the study of stress markers.

1.2 | Research Aim

The objective of this study was to attempt to provide a better understanding of the epidemiological distribution and morphological characterization of HLs to refine interpretations of the skeletal stress marker in bioarchaeology. With this intent, the study utilized clinical samples to avoid the biases of bioarchaeological research, such as the quantity and quality of skeletal preservation and contextual information.

Hypothesis 1. *Considering the characteristics of endochondral ossification (e.g., duration and vascularization) (Coqueugniot and Weaver 2007; Hughes et al. 1996; Ortner 2003; Park 1964) and the morphology of trabecular organization (Hughes et al. 1996), we expect to find a greater frequency of HLs along the lower limbs rather than the upper ones.*

Hypothesis 2. *An analysis of two samples from distinct contexts is expected to reveal a higher frequency of HLs in individuals from the more disadvantaged sociocultural setting (Papageorgopoulou et al. 2011). Moreover, applying the concept of the biocultural profile, HLs are hypothesized to serve as potential biocultural indicators (Beatrice et al. 2021; Beatrice and Soler 2016).*

Hypothesis 3. *As males are more susceptible to various diseases, in particular infections (females' biological buffering) and need a higher* calorie *intake during growth, we expect to find a higher frequency of HLs among men (*Kruger and Nesse 2007).

Hypothesis 4. *Considering the role of remodeling in the evanescence of HLs, we expect to find a lower frequency of these lesions among older individuals (Nowak and Piontek 2002b).*

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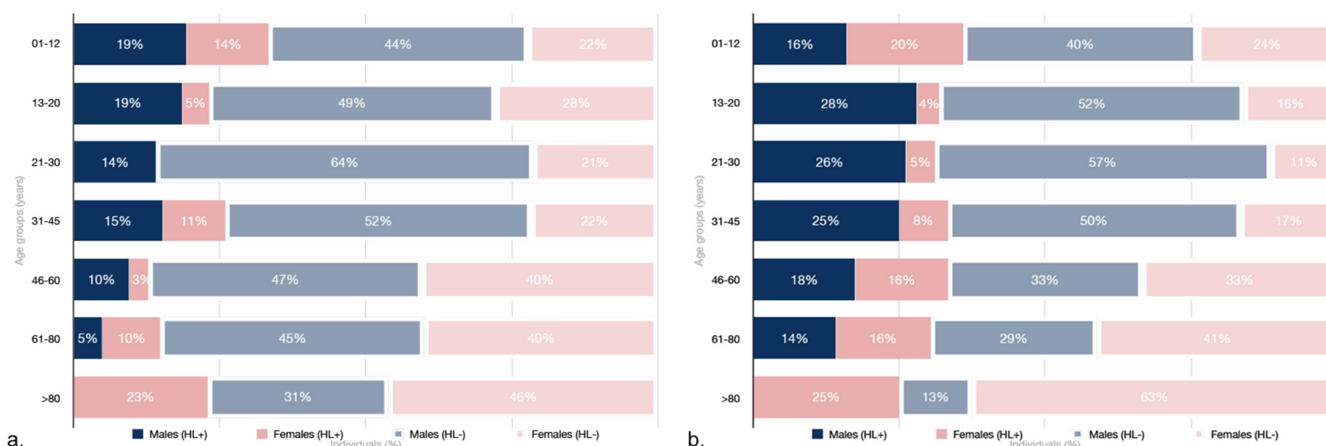

FIGURE 1 | (a,b) Distribution of individuals with HLs according to age groups. On the right side the Italian (a) sample, on the left the Sri Lankan (b). HL+: with Harris lines, HL-: without Harris lines.

2 | Materials and Methods

The definition and identification of Harris lines is not standardized and differs among authors (Ameen et al. 2005; Beom et al. 2014; Fiscella et al. 2008; Goodman and Clark 1981; Hummert and Van Gerven 1985; Mays, 1985; Piontek et al. 2001). For the purposes of this paper, we define HLs as radiopaque lesions located on the diaphysis or metaphysis of long bones (i.e., humerus, radius, ulna, femur, tibia, fibula), transversally orientated, and extended to at least halfway across the bone section (Hummert and Van Gerven 1985; Macchiarelli et al. 1994; Scott and Hoppa 2015). In this study, radiographs were oriented with anteroposterior and/or mediolateral projections, without any preference.

The distribution of HLs among different populations was investigated using conventional radiographs from two distinct samples. The first sample comprised 425 radiographs from 268 individuals (164 males, 104 females) collected from the IRCCS Galeazzi Hospital in Milan, Italy, while the second consisted of 834 radiographs from 293 individuals (185 males, 108 females) obtained from the Teaching Hospital in Jaffna, Sri Lanka (Department of Forensic Medicine and Pathology). Throughout the study, the first sample will be referred to as "IT" and the second one as "SR." Selection criteria mandated that individuals must have at least one long bone visible on radiographs, and that age and sex were known. All radiographs were captured using X-rays combined with a digital detector, and their examination was conducted by one of the authors (CM). The bias related to the lack of a standardized acquisition protocol was mitigated by removing all radiographs with unsuitable projections (e.g., overlap of bone) and parameters (e.g., overexposure, underexposure).

All data collected was anonymized and archived under alphanumeric labeling: "SR + serial n°" for individuals from Sri Lanka, and "IT + serial n°" for those from Italy. The study was conducted in accordance with the Declaration of Helsinki, and the retrospective evaluation of images from the Italian sample was approved by the Ethics Committee of Vita-Salute San Raffaele Hospital (RETRORAD protocol, Milan, Italy). Since the data were obtained retrospectively and the identifying details of the patients were not revealed, the ethical permission was obtained by the Deputy Director of the Teaching Hospital of Jaffna.

For statistical purposes, the ages of the individuals were classified into the following groups: 1–12, 13–20, 21–30, 31–45, 46–60, 61–80, >80 years, Adult ND. Statistical analyses were performed using Fisher's test, chi-square tests, and odds ratios, avoiding multiple testing. p Values <0.05 were considered significant. Moreover, on a subsample of 100 radiographs, the Cohen kappa test was performed to investigate the intra-observer (CM) and the inter-observer error (CM, LBG), with the guidelines from Landis and Koch (1977).

3 | Results

The Cohen's kappa value for inter-observer (*kappa = 0.887*) error reported a near perfect agreement with the two observers; therefore, the reproducibility of the observation is high. The same degree of agreement was present for the intra-observer reliability (*Cohen's kappa = 0.811*).

3.1 | Sri Lankan Sample

HLs were detected in 32% ($n=94$) of the Sri Lanka sample, comprising 293 individuals. Males showed a frequency of HLs of 63.8% ($n=60$; 20% of the total sample, 32.4% of males), contrasting with 36.1% for females ($n=34$, 12% of the total sample, 31.4% of females), although this difference was not statistically significant ($p=0.866$; Fisher test). It is noteworthy that males accounted for 63.13% of the assemblage ($n=185$) while females represented 36.8% ($n=108$) of the total sample. The highest frequency was observed among individuals aged 1–12 years (36% of individuals in this range) and 31–45 years (34% of individuals in this category) (Figure 1), though age was not a significant factor in the distribution of HLs (chi-square, $p=0.570$).

3.2 | Italian Sample

Within the sample of 263 individuals, 55 (20.4%) were identified as having HLs, with 60% being male (20.7% of males) and the remaining 40% female (20.1% of females), a difference that was not statistically significant ($p=0.915$; Fisher test). Similarly to the Sri Lanka findings, there were more males ($n=164$) than

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females ($n = 104$) in the total sample, and the peak frequency occurred among individuals aged 1–12 years (36% of individuals in this range) and 31–45 years (34% of individuals in this range) (Figure 1), though here age played a significant role in the distribution of HLs (chi-square, $p = 0.038$).

3.3 | Total Sample

HL distribution along the skeleton revealed a significantly higher frequency of HLs in lower limbs compared to upper limbs (chi-square test, $p < 0.00001$). However, it is important to note that the number of lower limb radiographs ($n = 388$ individuals) exceeded those of upper limbs ($n = 182$ individuals). A higher frequency of HLs was found on the tibiae (SR: 60.6%, IT: 94.5%) with males showing a higher frequency of HLs (30.3%) than females (24.5%) (SR, M: 36.2%, F: 25%; IT, M: 25%, F: 24.1%), but no statistical differences were found between females and males inter- or intra-sample (chi-square test, $p > 0.05$). The radius exhibited the second highest frequency of HL (9.89%), and a statistically significant discrepancy in HL frequency between females (16.3%) and males (6.61%) was detected (chi-square test, $p = 0.031$). The femur displayed a HL frequency of 4.38% among individuals of the sample, with a minimal difference between males (3.79%) and females (6.61%). Notably, the radius and femur exhibited anomalous patterns: both were highly represented in the Sri Lankan sample (radius: 14.6%; femur: 8.47%), while virtually absent in the Italian sample (radius: 0%; femur: 0.94%). The fibula (total: 2.31%, M: 2.95%, F: 1.32%) and ulna (total: 2.19%, M: 2.47%, F: 1.63%) yielded similar qualitative and quantitative data. In 0.17% of cases, HLs were detected on the humerus, and only in males (Table 1). Based on descriptive statistics, the predominant side was the right for femora (58%) and humeri (100%), while no distinctions emerged for the other long bones. Only 3.35% of individuals with HLs displayed symmetric lesions ($n = 6/149$). Of these, 66.6% were females aged between 39 and 81 years; and the rest (33.4%) were males with an age of 55 to 58 years. Considering all cases, 57% of symmetry was recorded on the tibia, 14% on the radius, 14% on the femur, and 14% on the fibula.

A significant disparity between IT and SR samples was identified (chi-square, $p = 0.001$), with a prevalence of HLs among SR individuals. Moreover, individuals from Sri Lanka had an odds ratio of 1.83 to manifest HLs. Statistical analysis indicated that the variation in HL distribution between females and males is not significant (chi-square, $p = 0.796$). Nonetheless, a significant difference was observed between IT and SR males (chi-square test, $p = 0.013$), whereas the distribution among females did not yield the same result (chi-square test, $p = 0.060$).

The median age was determined to be 39 years in the SR sample, 32 years in the IT sample, and 39 years in the total sample. A linear trend was not found in the distribution of HLs among age groups. In fact, in the SR sample, the frequency of individuals with HLs in the 86–91 year group resembled that of the 51–55 and 36–40 year groups. Moreover, males presented a higher frequency of HLs between 21–40 years, with a progressive decrease from the age of 65, while women showed higher frequencies from age 51 onwards. In the IT sample, a similarity was noted between elderly individuals (86–92 years) and juveniles (11–20 years), although the sample itself was characterized by a decrease in the number of individuals in certain age categories (21–25 years: 14 individuals; 81–85 years: 4 individuals) (Figure 2).

4 | Discussion

4.1 | Distribution of HLs Along the Skeleton

The first hypothesis of this paper can be accepted as a lower frequency of HLs was observed among upper limbs. This result could be related to twisting and bending movements of the arms, which may facilitate a constant and more intense bone turnover compared to the lower limbs (Harris 1931). Some authors suggested the opposite hypothesis (Gupta et al. 2006; Tsubota et al. 2002) but there is no clinical confirmation for this (Bennell et al. 1997; Karlsson et al. 2003). Although marginal, another contributing factor to this distribution could be the timing of bone maturation (Coqueugniot and Weaver 2007; Hughes et al. 1996; Jit and Kaur 1989; McKern and Stewart 1957; Sahni et al. 1998; Scheuer and Black 2004). Lower limbs might be more prone to developing HLs because of a higher concentration of red blood cells (via vascularization) and osteoblasts, which could be involved in the infections response (Boucherie et al. 2017; Hughes et al. 1996). The higher frequency found along the tibiae is consistent with prior research, supporting the selection of this bone for sampling, as the distribution of HLs on tibiae is not influenced by sex on the present sample (Beom et al. 2014; DeWitte and Slavin 2013; Grolleau-Raoux et al. 1997; Hughes et al. 1996; Piontek et al. 2001; Primeau et al. 2018b). It is likely that the higher frequency of HLs on the tibiae is due to the extensive vascularization during growth and the concentration of osteoblasts, increasing the susceptibility of this bone to develop responses from environmental stressors (Hughes et al. 1996; Park 1964). However, the bias related to this sample (e.g., the imbalance in the availability of radiographs between lower and upper limbs, and the difference in limbs projections) could have impacted the visibility of HLs. Therefore, the higher frequency of this lesion along tibiae could be due to the sample itself. With the aim to limit this bias, we analyzed the frequencies of HLs for each bone, rather than for the total sample (i.e., the sum of all elements considered). The distal portion of the humerus and the proximal portion of the femur are less subject to the development of lesions compatible with the definition of HL since the growth plate is not perfectly transverse, unlike the tibia (Hughes et al. 1996). The higher frequency of HLs was observed in the distal portion, although it must be noted that different radiographic projections were analyzed, with not all presenting both the proximal and distal portions of the same bone. While acknowledging this bias, the result may be attributed to the growth pattern of the tibia, with approximately 57–60% of elongation occurring proximally and 43–40% distally (Allison et al. 1974; Byers 1991; Kulus and Dąbrowski 2019). Given that both epiphyses fused between 14 and 20 years (14–18 years for females, 16–20 years for males) (Scheuer and Black 2004), it is plausible that the growth rate differs between the two portions, with osteoblastic activity being more intense proximally. Furthermore, the cortical bone of distal tibiae is thinner than in the diaphyseal

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TABLE 1 | Distribution of HLs across the skeleton.

Sri Lanka					Italy				
Bone	***n/n***	***%***	***n M and F***	***% M % F***	***Bone***	***n/n***	***%***	***n M and F***	***% M % F***
Tibia	57/293	19.4%	41/185 (M), 16/108 (F)	22.1% (M), 14.8% (F)	*Tibia*	52/268	19.4%	31/164 (M), 21/104 (F)	18.9% (M), 20.1% (F)
Femur	15/293	5.11%	7/185 (M), 8/108 (F)	3.78% (M), 7.40% (F)	*Femur*	2/268	0.67%	2/164 (M)	1.21%
Fibula	2/293	0.68%	1/185 (M), 1/108 (F)	0.54% (M), 0.92% (F)	*Fibula*	7/268	2.61%	6/164 (M), 1/104 (F)	3.65% (M), 0.96% (F)
Humerus	1/293	0.34%	1/185 (M)	0.54% (M)	*Humerus*	0/268	0%	0/	0%
Radius	18/293	6.14%	8/185 (M), 10/108 (F)	4.32% (M), 9.25% (F)	*Radius*	0/268	0%	0/	0%
Ulna	4/293	1.36%	3/185 (M), 1/108 (F)	1.62%(M), 0.92% (F)	*Ulna*	0/268	0%	0/40	0%

Total sample

Bone	*n/n total*	*%*	*n M and F*	*% M % F*
Tibia	109/561	19.4%	72/349 (M), 37/212 (F)	20.6% (M), 17.4% (F)
Femur	17/561	3.03%	9/349 (M), 8/212 (F)	2.57% (M), 3.77% (F)
Fibula	9/561	1.60%	7/349 (M), 2/212 (F)	2% (M), 0.94% (F)
Humerus	1/561	0.17%	1/349 (M)	0.28% (M)
Radius	18/561	3.20%	8/349 (M), 10/212 (F)	2.29% (M), 4.71% (F)
Ulna	4/561	0.71%	3/349 (M), 1/212 (F)	0.85% (M), 0.47% (F)

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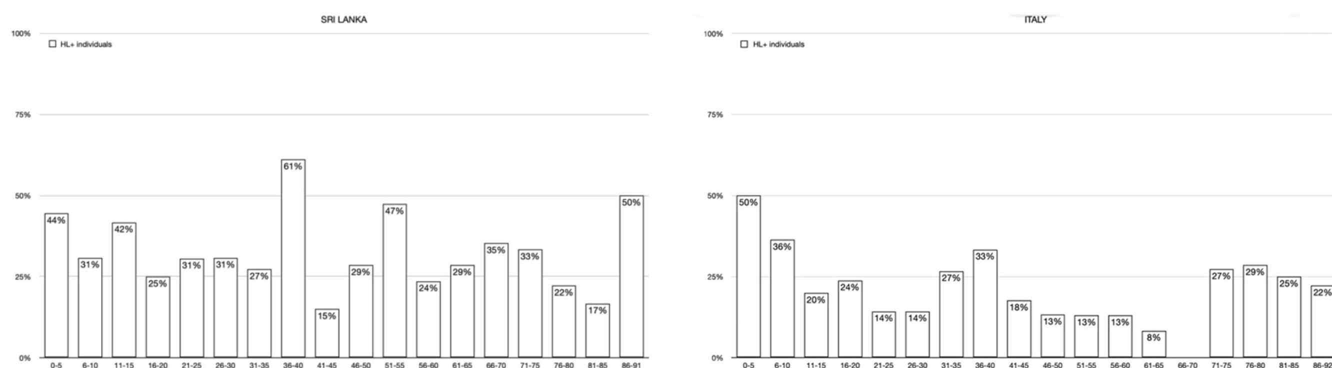

FIGURE 2 | Distribution of HLs according to age groups.

portion, facilitating HL detection (Garn and Braunstein 1986). HLs were more frequent on the right side exclusively in femora and humeri, while in fibulae they were evenly distributed between sides. Hughes et al. (1996), who also noted a predominance of HLs on the left side, suggested that lateralization of HLs might stem from localized remodeling focused on areas experiencing greater mechanical load, such as the right forearm. Fiscella et al. (2008), albeit in a smaller sample ($n = 24$), similarly observed a higher frequency of HLs on the left side. No significant differences in age and lateralization were found, contrary to Hughes et al. (1996), who observed a higher frequency of HLs on the right side among tibiae and fibulae of younger individuals (0–24 years).

The low frequency of symmetric lesions, conversely to the results of Inokuchi et al. (2000), may be attributed to the heterogeneity of the radiographs, as not all individuals underwent bilateral X-ray analysis. This bias could compromise interpretation, as symmetric lesions have been used to validate the accurate classification of HLs among stress markers.

The present findings could serve as an additional tool for designing sampling strategies in archeological and clinical studies. Indeed, the absence of taphonomic alterations (while common in archeological samples) could provide a more accurate estimation of where HLs might be observed. Specifically, the sampling method proposed suggests prioritizing tibiae due to the high frequency of HLs observed, as evidenced by Hughes et al. (1996), and the absence of a significant association with biological sex. Moreover, tibiae have long been favored for sampling, and maintaining and validating past sampling methods promotes standardized practices and facilitates cross-study comparisons.

4.2 | Differences Between Geographical Samples

The difference between SR and IT samples was reflective of the different lifestyles and access to medical care and food between the two countries, thereby affirming the role of HLs as stress markers rather than physiological lesions. This result confirms the second hypothesis of this research. Indeed, the gross domestic product of the two countries (Sri Lanka: 3.354 USD; Italy: 34.776 USD[1]) shows a clear disparity which may be seen as a reflection of their difference in standard of living. The civil war in Sri Lanka, which lasted until 2009 (1983–2009), had a detrimental effect on the economy and the lifestyle of the population, a situation that was further exacerbated by mass catastrophes. Post-World War II Italy, on the other hand, experienced a state of political and economic crisis that was resolved in the 1960s. From this point onwards, there was growing economic and social welfare, which also had repercussions for healthcare (Banti 2009). The socioeconomic contexts of the two samples under consideration are thus found to be significantly divergent. While a steady economic recovery is observed in one sample, a decrease of wealth is noted in the other. It can thus be hypothesized that individuals who were raised in these two contexts may have been exposed to divergent environmental stressors, with the individuals from Sri Lanka experiencing more limited access to food and health resources.

Both in Italy (current sample) and the United States (Garn and Schwager 1967), 20% of individuals exhibited HLs, suggesting that in these contexts, HLs could emerge as a consequence of medical interventions (e.g., surgical operations, increased survival rates from infections, and overall stress) (Garn et al. 1968). It is therefore important to pay attention to the environmental, cultural, and socioeconomic context of origin of the individuals under study before advancing an interpretation. Indeed, HLs are adaptive responses resulting from an individual's stress; however, caution must be exercised in interpretations, especially when comparing two distinct contexts.

The odds ratio calculated (SR sample: 1.83 odds ratio, $p = 0.001$; IT sample: 0.55 odds ratio, $p = 0.001$) illustrated how, for the first time, HLs could be integrated into the biocultural profile. The biocultural profile aims to consider skeletal signs of biological adaptation to social, cultural, and environmental experiences to distinguish undocumented border crossers (coming from Central America) from American-born individuals (Beatrice et al. 2021; Reineke et al. 2023). This emphasizes that the embodied stress might be relevant to recognize migrants, following the structural violence approach. Specifically, this approach could be useful in forensic and bioarchaeological practice to recognize groups from divergent socio-economic backgrounds, as well as to identify structural violence (Beatrice et al. 2021; Beatrice and Soler 2016; Biehler-Gomez et al. 2023; Birkby et al. 2008; Weisensee and Spradley 2018). However, the essential requirement for its correct interpretation is knowledge of the context of growth of the individuals (Reineke et al. 2023). Moreover, identifying and isolating the most characteristic stress markers could aid in comprehending how structural violence manifested in different contexts, potentially informing applications in

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bioarchaeological settings. This study sought to determine if the presence of HLs could serve as a discriminating factor between IT and SR samples, and the result corroborated this hypothesis.

4.3 | Differences Between Sexes

The third hypothesis of this study can be rejected as HLs were not more frequent among males. However, the distribution of the lesion mirrored the societies analyzed, which were not characterized by sexual disparities in access to food resources and healthcare.

The slight predominance of men (26.9%) over women (25.9%) with lesions may be linked to differences in bone maturation or to the female biological buffering (chi-square, $p = 0.796$) (Kruger and Nesse 2007). Indeed, males' bone growth proceeds slower, allowing a broader time frame for embodying stress. Despite the fact that differences are slight and confined to a couple of years (Scheuer and Black 2004), this variation might have an impact on HL formation. Moreover, this physiological difference should be credited when the period of formation of the lesion is described: peaks at ages correlated to the *saltation* phase differ between males and females. The higher susceptibility of men to embody environmental stress by displaying stress markers in skeletal samples was not corroborated in studies on HLs nor in the present research (Goodman and Rose 1991; Stinson 1985). In the present population, males and females were exposed to the same environmental and social stressors: access to resources was relatively equal and no major disparities in medical services were recorded. Following the *female buffering* theory, in a similar context males should show a higher frequency of HLs, but the results showed a different trend. A comparable distribution of HLs between the two sexes (Ameen et al. 2005; Primeau et al. 2018a), as well as an opposite outcome (Beom et al. 2014; Biehler-Gomez et al. 2025; Garn and Schwager 1967; Gindhart 1969; Hummert and Van Gerven 1985; Papageorgopoulou et al. 2011; Piontek et al. 2001; Velasco-Vázquez et al. 1999), are often observed in archeological samples. The differences observed in these studies might, therefore, reflect a different lifestyle between females and males.

The divergence between SR and IT females, and SR and IT males could reflect exposure to different environmental stressors, with SR males potentially experiencing harsher lifestyles compared to their Italian counterparts. In light of this evidence, the similarity between sexes observed in the total sample (M: 26.9%, F: 25.9%) could not be attributed to physiological growth (Alfonso et al. 2005), as otherwise, the discrepancy between IT and SR males would not have been evident.

4.4 | Age Distribution

As posited in bioarchaeological studies (Nowak and Piontek 2002b), the evanescence of HLs is attributable to bone remodeling. Nevertheless, the distribution of this lesion in the present sample does not demonstrate a decrease with age; therefore, the fourth hypothesis of this paper can be cautiously rejected. Based on this result, and with appropriate caution, this suggests that ageing and bone turnover might not be the primary agents responsible for the disappearance of HLs. This finding presents a new perspective on the distribution of HLs, although further investigations and alternative methodological approaches would be necessary to validate this trend (e.g., histological analysis).

However, the absence of osteocytes (e.g., cells involved in bone remodeling) recognized in histological studies on HLs (Miszkiewicz 2015) could support our results. Furthermore, the untargeted remodeling responsible for HLs evanescence was defined as a physiological process, and it would be methodologically incorrect to attribute a non-pathological process to a pathological lesion (HL). In addition, it should be noted that homeostasis and the static balance of trabeculae alter their orientation over the years. Therefore, if HLs were perceived as biomechanical obstacles, their remodeling would have been targeted, rather than persisting into old age. Moreover, the hypothesis of HL remodeling was primarily proposed based on bioarcheological studies, which typically involve samples scarcely composed of elderly individuals. It may be more appropriate to discuss a reduction in the thickness of lesions rather than their complete disappearance, as the latter is rarely observed in other pathological processes (such as callus remodeling). While it is known that bone density decreases after the age of 50 due to the imbalance between osteoclastic and osteoblastic activity (Garn et al. 1992), this physiological change may not be directly related to the presence of HLs.

HLs in elderly individuals in this sample could be related to lifestyle factors. For instance, in Europe, living conditions were harsher 80 years ago (e.g., World War II), whereas access to medical care and food resources has significantly improved in recent years. This generational difference is reflected in the distribution of HLs and confirms their role as stress markers. If HLs were considered physiological lesions, a consistent correspondence between young and older individuals would be expected in the Italian sample.

4.5 | The Morphology of Radiopaque Lesions

The lack of a standardized definition of HLs presents a potential obstacle to the comparison between studies and may introduce variability in the observations themselves. The term "Harris lines" is, in fact, used to indicate a broad category of lesions, which differs by morphological features and etiological factors. The recording standard is based on antero-posterior radiographs, traditionally preferred in bioarcheological studies, and does not accommodate the analysis in different projections, which are very common in living individuals (Scott and Hoppa 2015). Moreover, the sampling criteria (transverse extension, inclination, and contact with cortical bone) differ among authors, leading to a possible misinterpretation of the lesions, and biasing the comparison between studies. For instance, the extension of radiopaque lines has been documented to vary from one quarter across the bone width (Ameen et al. 2005; Beom et al. 2014; Fiscella et al. 2008; Goodman and Clark 1981; Piontek et al. 2001) to half (Hummert and Van Gerven 1985; Mays, 1985).

This research evidenced a great variability in transverse radiopaque lesions, which morphology could be related to

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different etiologies, thereby providing valuable tools for the study of past populations (Table 2 [only online], Table 3 [only online]). As the individuals' medical histories were not provided, consistent correlations between etiological factors and specific lesions' morphology could not be proposed. Yet, in current literature, various morphologies were identified, including HLs, zebra lines, Frenkel lines, bone trauma recovery lines, and lead lines (Al Muderis et al. 2007; Georgiadis and Gannon 2022; Golriz et al. 2017; Jacobson and Lee 2012; Jaramillo et al. 1990; Leone 1968; Polat et al. 2015; Zapala et al. 2016).

4.5.1 | Case Studies

Here, we propose to investigate the morphology of transverse radiopaque lines to improve interpretation of these defects from three case studies. However, due to the absence of anamnestic data, it was not possible to propose a specific etiology for the radiopaque lesions under investigation. Therefore, the interpretation suggested below must be considered hypothetical and not conclusive.

4.5.2 | Metabolic Conditions and Nutritional Deficiencies

Scurvy has been clinically defined as a condition characterized by a concentration of ascorbic acid lower than 23 μmol/L in the body. Instances of this pathology persist, albeit with relatively low frequency, even in the richest geographical areas, such as the United States (Golriz et al. 2017). Therefore, the sample under consideration may potentially include individuals suffering from scurvy. Radiographic manifestations associated with this condition include Wimberger's ring (a radiopaque area that surrounds the perimeter of the fused epiphysis), Trummerfeld area (a radiopaque area in the metaphysis), Pelkan fracture (localized in the metaphyseal area and characterized by lateral bone deposition), and Frenkel's line (morphologically very similar to HLs, appearing near the distal end of the bone and exhibiting a trajectory that follows the shape of the metaphyseal line) (Golriz et al. 2017; Polat et al. 2015). Additionally, scurvy manifests osteologically through pronounced periosteal reactions, which are often challenging to discern on radiographs (Brickley and Ives 2008).

Although comprehensive anamnestic data regarding the individuals were unavailable, one case (IT–167, a 7-year-old male) presented radiopaque lesions with a peculiar morphology. In fact, the alignment of these lesions deviated from perfect transversality, instead appearing to follow the contour of the growth plate. While reminiscent of Frenkel lines, this limited radiographic analysis was not enough for a definitive diagnosis. Indeed, the absence of other lesions, clinical/anamnestic evidence, and comprehensive radiographic assessment constrained diagnostic certainty. Accordingly, our focus lay in emphasizing the morphological variability of the striations and advocating for further investigation on this topic. Enhanced clarity regarding the defining characteristics of Frenkel lines would facilitate their recognition and enable more nuanced differential diagnoses in interpreting such striations (Figure 3).

4.5.3 | Intoxications

Lead intoxication can induce radiopaque bands along the growth plate of juvenile individuals, albeit with a thickness exceeding that of HLs. These lead lines are not caused by an interruption in growth but rather from the accumulation of calcium and lead (Caffey 1931; Hughes et al. 1996) within the bone matrix (Leone 1968). However, no individual in the present sample manifested lesions comparable to those produced by lead poisoning. Another type of "intoxications striation," referred to as zebra lines, shares similarities with HLs but differs in etiology and persistence. In fact, zebra lines typically disappear within a few years (3–4 years) following cessation of bisphosphonates treatment, whereas HLs may persist for decades (Al Muderis et al. 2007).

Individual IT–168 (6-year-old male) underwent two X-ray sessions spaced 1 month apart. During the second session, a series of radiopaque striations along the proximal portion of the right tibia were noted, exhibiting distinct characteristics in terms of spacing and thickness. These lines appeared within an already mineralized bone area, incompatible with HLs pathogenesis but consistent with medication effects. The most proximal lesions could be categorized as zebra lines, which are not related to growth interruptions (Loizidou et al. 2017). Bisphosphonates, by inhibiting osteoblastic activity, disrupt the remodeling process of the primary spongiosa, leading to the formation of these radiopaque lines (Al Muderis et al. 2007; Van Persijn van Meerten et al. 1992). While

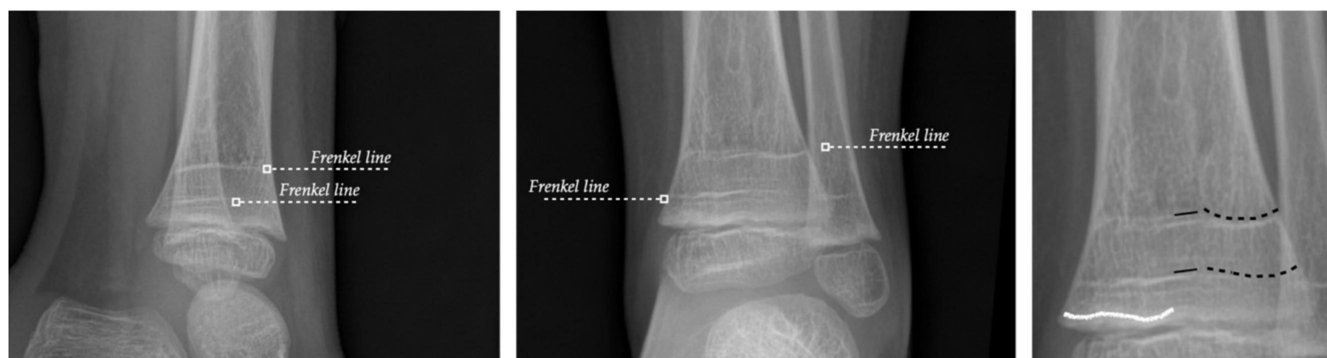

FIGURE 3 | The radiopaque lesions observed in both fibula and tibia (individual IT–167) are located in the metaphysis and share the sample orientation of the growth plate. Those characteristics are consistent with the lesions named Frenkel lines.

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the absence of medical records precludes confirmation of their diagnosis as zebra lines in individual IT–168, a morphological evaluation enabled differentiation of these lesions from HLs, thereby proposing a more complex interpretation of the present case. The identified HLs could alternatively be interpreted as "migrated" zebra lines, although their movement tends to be directed towards the metaphyses rather than the center of the diaphysis (Van Persijn van Meerten et al. 1992) (Figure 4).

4.5.4 | Trauma

Radiopaque lesions typically form from 6 to 12 weeks following a traumatic event and may not be perfectly transverse, sometimes not exceeding 25% of the medullary cavity (Cepela et al. 2016; Ecklund and Jaramillo 2002; Hynes and O'Brien 1988; Jacobson and Lee 2012; Ogden 1981; Park 1964; Rang 1968; Salter and Harris 1963). Trauma limited to the metaphyseal area during growth may lead to the formation of radiopaque lesions due to involvement of the growth plate.

Individual IT–140 (44-year-old male) underwent metal element removal, and 6 days post-procedure, radiopaque lines became apparent precisely in correspondence with the site where the nails were inserted. These lesions could be categorized as "recovery lines," as they do not arise from growth interruption (Cepela et al. 2016; Hynes and O'Brien 1988; Ogden 1981; Park 1964; Rang 1968; Salter and Harris 1963). While the position of the recovery lines coincided perfectly with the nails, the relatively short interval between the two X-rays seemed insufficient for the development of these lesions. Given their mode of formation, it would be pertinent to investigate their relationship with bone remodeling and the biomechanical stability of trabeculae (Figure 5).

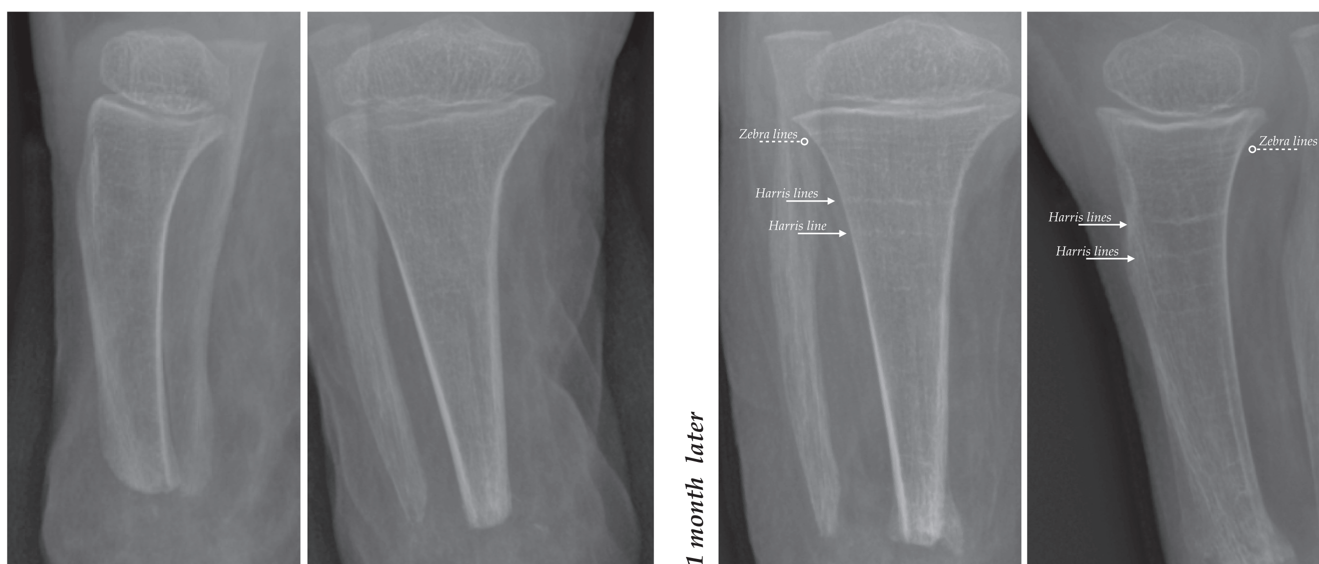

FIGURE 4 | The tibia of the same individual (IT–168) was radiographed again after a one-month interval (figures on the right), and four radiopaque lesions were observed. The wavy orientation and the expansion over the half of the diameter of the two lesions located in proximity of the growth plate are consistent with the morphology of the zebra lines. The characteristics of the other two lesions (e.g., transverse orientation, expansion over the 25% of the diameter) is compatible with Harris lines.

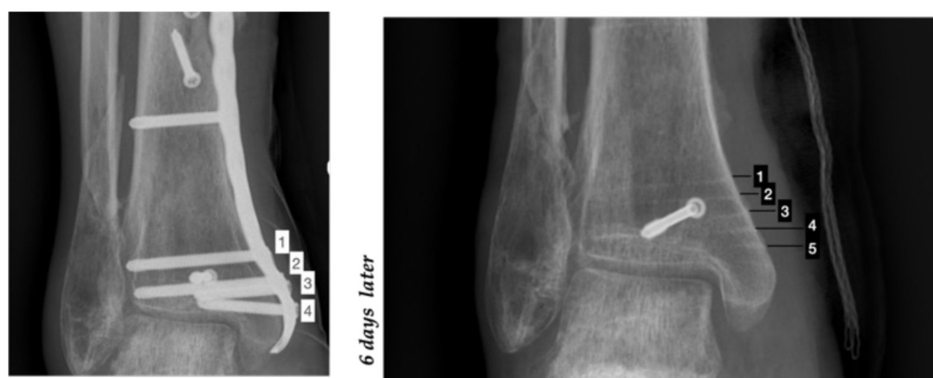

FIGURE 5 | The tibia and the fibula of the same individual (IT–140) was radiographed again after a 6-day interval (figures on the right). In the recent radiographs, the appearance of five transverse radiopaque lines is observed. The morphology of these lesions (e.g., orientation similar to metal nails) and the correlation with the surgical procedure of metal elements remotion are consistent with the interpretation of these sings as recovery lines.

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5 | Limits

The study conducted on a clinical sample encountered several limitations. The major bias was related to the lack of standardized acquisition protocols, due to the different provenance of the sample. In fact, the projections for radiographic analysis were not equal for the two samples, as well as the parameters (kV, mAs), which were not available. Moreover, the presence of prostheses and fractures disturbed the penetration of X-rays. In this paper, 61 individuals (10.8%) had underexposed radiographs. Furthermore, the presence of plasters or thick gauze further complicated radiographic interpretation. Prostheses were identified in 97 subjects (17.2%), obstructing visualization of the medullary cavity, and 4.6% (26 individuals) showed bone fractures that obscured the medullary cavity. To mitigate factors of error, reliability tests (inter-observer and intra-observer) were conducted. Moreover, the analysis of the radiographs was carried out by a single operator (CM) and repeated twice to ensure consistency and reduce the potential for eye fatigue-induced errors. All non-legible radiographs were excluded from the sample, retaining only the images in which HLs could have been observed.

The most frequent orientation was antero-posterior; however, the use of different projections hindered the establishment of a standardized database and systematic analysis of bones. Moreover, the imbalance in the availability of radiographs between lower and upper limbs must be acknowledged as a limit of the sample. To mitigate this bias, as well as the different standardized acquisition protocol of the two samples, all the illegible radiographs were excluded from the sample (e.g., overlap of bone).

The absence of anamnestic data and infrequent repetition of radiographs over time impeded investigations into the etiology of the radiopaque lesions and the role of remodeling in their evanescence.

Lastly, the lack of data about the age at formation of HLs hinders the capacity for an etiological differentiation of the lesions. For instance, all the lesions formed during the period of growth saltation cannot be recognized.

6 | Conclusion

When sampling is needed and limited to specific skeletal areas, knowledge of preferential sites could facilitate and standardize procedures (Boucherie et al. 2017).

The results on the distribution of HLs along the appendicular skeleton allow us to propose a series of practical recommendations for analysis in contexts where full-body X-rays are unfeasible. The proposed sampling strategies can be summarized in the following points: lower limbs, particularly tibiae, should be preferred, while the humerus should be avoided due to ambiguous trabecular composition; the radius and femur present high frequencies of HLs but seem to be influenced by biological sex; sampling priority should be given to the tibia, femur, and fibula for the lower limbs, and radius, ulna, and humerus for the upper limbs; finally, the left side is preferable for sampling.

The presence of HLs in the Italian sample, representing a relatively wealthy context with ample access to food resources and characterized by a healthcare system capable of dealing with infections and milder pathologies, prompts reflection on the significance attributed to these lesions. This does not question their role as stress markers but rather underlines how their interpretation in a similar context may reflect access to healthcare and the role of micronutrients in bone development. Statistical analysis supported the inclusion of HLs into the "biocultural profile" in this sample, as they showed a strong association between HLs and the SR sample.

No sexual disparities in the distribution of HLs were observed overall, reflecting the structuration of the society with regard to food access and medical care. However, a significant difference was observed among males in the SR sample, suggesting potential internal disparities.

Physiological bone turnover should, with the increasing age, concur with the evanescence of these lesions. This reasoning is supported by bioarcheological studies (Nowak and Piontek 2002b); however, an opposite trend is observed in the current research. In fact, contrary to expectations, HLs were found among older individuals. Hypothetically, this dichotomy might be related to a low frequency of elder individuals in archeological populations or to a poorer impact of bone turnover in HL evanescence than expected. However, without a systematic histological analysis, this trend cannot be confirmed.

The diverse morphology of transverse radiopaque lines found underscores the need for a standardized definition of HLs and an in-depth analysis of its variations to allow for etiological differentiation.

Though preliminary, this study presents several potential avenues for further exploration through additional analyses. Specifically, examining the role of bone remodeling, correlating with biogeographic origin, exploring morphological variability, and considering independence from sex all open new perspectives regarding the further applications and interpretations of HLs in clinical, bioarchaeological, and forensic contexts.

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Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Endnotes

[1] Data from World Bank (https://datacatalog.worldbank.org/search/dataset/0037712).

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Harris lines and growth plates. **Table S2:** Classification criteria of radiopaque lines. **Table S3:** Tabulated summary.

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