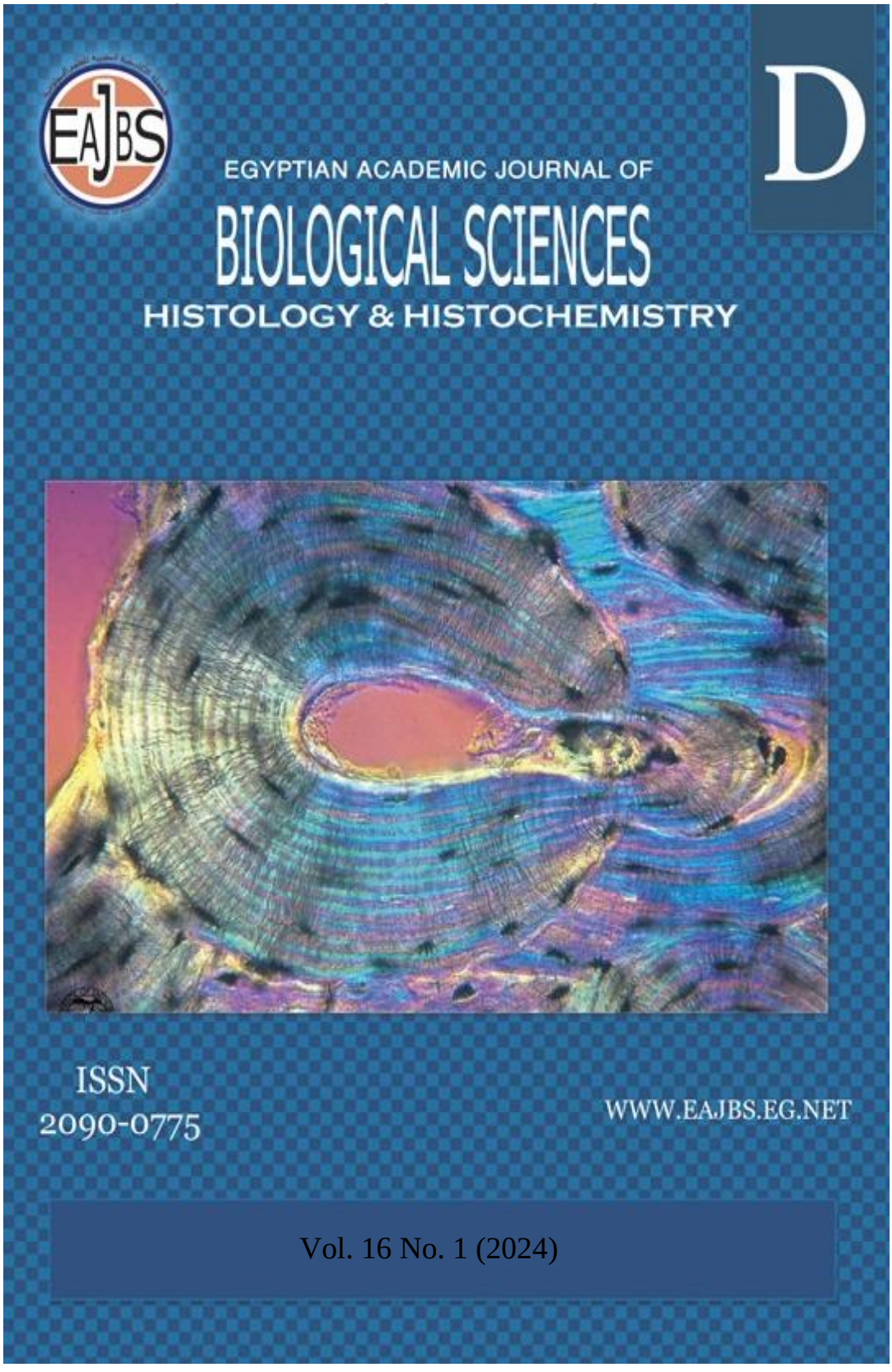

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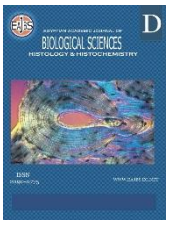

Helicobacter pylori (HP) Infection-Associated Modulation in The Bone Profile of HP-Infected Individuals

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ABSTRACT

Background: Extra-gastric implications of *H. pylori* infection have raised serious health concerns. The association of several extra-gastric diseases with *H. pylori* infection has been studied, in the recent past, however, information is lacking on *H. pylori*-associated modulation in the bone profile of the infected patients that may lead to bone disorders. The study aimed to assess *HP*-associated modulation in the vital components of the bone profile. **Methods:** A retrospective (hospital-based case-control) research (n = 385) was undertaken. *H. pylori*-associated alteration in the vital component of the bone profile was ascertained by Chi-square, V_{Cramer}, Bayesian V_{Cramer}, bias factor/log (BF_{01}), and logistic regression using R-packages. $P < 0.05$ was the cut-off for significance. **Results:** The difference in mean ± SD (female vs male) for uncorrected calcium (2.2 ± 0.3 vs 2.3 ± 0.3; p <0.001), albumin (37.7 ± 7.8 vs 40.7 ± 6.2; p <0.001), and alkaline phosphatase (69.3 ± 26.8 vs 77.1 ± 35.7; p = 0.016) was statistically significant. A significant difference in mean ± SD of corrected calcium of *HP*-infected vs *HP-un*infected was measured (2.17 ± 0.3 vs 2.19 ± 0.3; p = 0.04). The proportion of hyper/hypo-calcemia, hyper/hypo-magnesemia, hyper/hypo-phosphatemia, and hyper/hypo-albuminemia was independent (no association) of *HP*-infection ($V_{Cramer} \approx 0.00$, log (BF_{01}) >100, $p > 0.5$). The association of only hyperphosphataemia (AOR: 2.68; CI: 1.18–6.13; $p < 0.05$) and hyperalbuminemia (AOR: 1.25; CI: 0.47–3.35; $p < 0.001$) with *HP*-infection was significant, however, *HP*-infection-dependent modulation in other components of the profile was not significant (p> 0.05). **Conclusion:** The association of only hyperphosphatasemia and hyperalbuminemia with *HP* infection was significant, however, *HP*-infection-dependent modulation in other components of the profile was not significant. Large group well-controlled research is recommended to gain comprehensive insight into it.

INTRODUCTION

H. pylori, a microaerophilic and ubiquitous bacterium (Goodwin 1993) infects ≈50% of the population of the world (A Gravina *et al.*, 2018). *HP* infection is hyper-endemic in Saudi Arabia affecting individuals across age groups (Al-Akwaa 2010). HP colonizes to incapacitate gastric mucosa and triggers gastrointestinal tract (GIT) related diseases (Cover & Blaser 2009; Khoder *et al.*, 2019).

Primarily, HP has been recognized as one of the major etiologies of a wide range of abdominal conditions and diseases (stomach ulcer, antrum gastritis, and stomach malignancies) (Blaser 1999; Carrasco & Corvalan 2013; Censini *et al.* 2001; Uemura *et al.*, 2001; Yeh *et al.*, 2009). Moreover, extra-gastric sequelae (pre-eclampsia, prostatitis, myocardial infarction, iron-deficiency anemia, hepatic disease, encephalopathy, and autoimmune disorders) of HP infection have also been reported extensively in the meta-analysis literature (Abdollahi *et al.*, 2016; Blecker *et al.*, 1991; Campuzano-Maya 2014; A Gravina *et al.*, 2018; Rahmani *et al.*, 2017). HP infection has been reported to indirectly affect bone turnover (resorption and formation) which may lead to the enhanced risk of bone disease such as osteoporosis (Wang *et al.*, 2019). HP infection-triggered systemic inflammation and cytokines generation namely tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-1 (IL-1) (Noach *et al.*, 1994) could be the possible mechanism behind the HP-associated alteration in bone turnover in the infected individuals (Wang *et al.*, 2019). Enhanced release of these inflammatory cytokines in HP-infected patients (Chung *et al.*, 2015) has been reported to be involved in declined bone mineral density (BMD) which may impact overall bone density and thereby affect the health of bones in the HP-infected individuals (Wang *et al.*, 2020). Recent studies have highlighted the association of HP infection with the major component/s of the bone profile (serum calcium, serum phosphorous, alkaline phosphatase, serum magnesium, serum albumin, and corrected calcium) of HP-infected patients (Wang *et al.*, 2020; Wang *et al.*, 2019). Serum calcium (Ca) occurs in 3 major forms: albumin-bound, lactate-bound, citrate-bound, and unbound/ionized calcium (iCa) (Peacock 2010). HP-induced hypochlorhydria and atrophic gastritis interfere with calcium absorption (Asaoka *et al.*, 2014), and calcium homeostasis which leads to affecting bone mass and osteoporosis (Kakehasi *et al.*, 2009). Kim et al reported the HP-associated osteoporosis in a previous study (Kim *et al.* 2021). Circulating Mg exists in bond (with plasma protein) and biologically active form (Baradaran & Nasri 2006). HP survival, virulence, secretion, and regulation of gastric acid are cation (Mg^{2+} and Ca^{2+})-dependent, and the association of HP infection with varied serum Mg levels has also been reported in a few studies (Nasri 2007). Two key isoforms of tissue-non-specific alkaline phosphatase (ALP) isozyme forms are bone-specific ALP (BALP) and liver-specific (LALP) which are found in serum in approximately equal proportion (Tariq *et al.*, 2019). Serum phosphate (P), Ca, and ALP are potent predictors of BMD (Shu *et al.*, 2022), and bone loss (Deftos *et al.*, 2001; Goretti Penido & Alon 2012; Thio *et al.*, 2020). P and Ca deficiency may eventuate in bone pathology (Goretti Penido & Alon 2012; Marks *et al.* 2010). Raised ALP has been reported to be correlated with HP infection (Gong *et al.*, 2015). HP infection alters the phosphate absorption by interfering with the duodenal pH because P absorption by the paracellular pathway operates effectively at acidic pH (Korucu *et al.*, 2020). To the best of my understanding, scientific information is lacking on the association of HP infection with a component/s of the bone profile, and thus the current study aimed to assess the HP-infection-dependent alteration in the vital components of the bone profile that is necessitated to articulate the policy for future research and management of bone health in the HP-infected patients.

MATERIALS AND METHODS

Ethical Approval and Declaration:

The Declaration of Helsinki (guidelines) was adhered to for carrying out this research. The study received ethical approval from the Research Ethical Committee (REC) constituted at Armed Forces Hospital, in the Southern

region, KSA. Approval details: reference code; AFHSRMREC, reference number; 2023/687, and approval date: 02/04/2023. The Research Ethical Committee (REC) has its policy for data protection and retaining the confidentiality of the data. In this study, data was secondary which was generated by routine laboratory investigations in the hospital. The purpose of the collection of data was explained through a proposal on the association of HP infection with extra-gastric disease before the committee.

Data Retrieval, Study Area and Population, Research Design, and Study Period:

A hospital-dependent case-control research design was implemented to assess the HP-infection-associated modulation in the components of the bone profile. Data was retrieved from laboratory investigation reports (secondary data generated by routine laboratory tests) (period 2017 to 2023) available at the respective hospital following inquisitive review and screening for demographic (gender and age) characteristics, bone profile, and other laboratory parameters (hematological parameters). The data was reviewed during the collection process. A comprehensive illustration of the methodological approaches followed in this research is depicted in Figure 1.

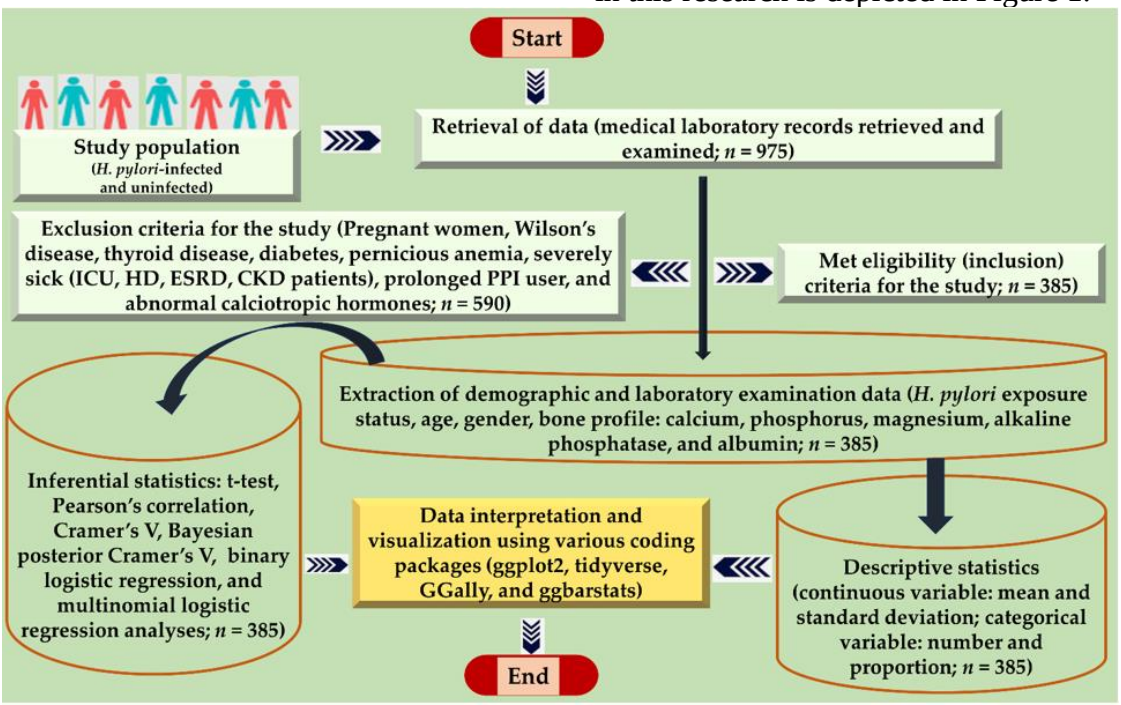

Fig. 1. Schematic illustration of methodological approaches for this research; *n* = number, *H* = *Helicobacter*, ICU = intensive care unit, HD = haemodialysis, ESRD = end-stage renal disease, CKD = chronic kidney disease, and PPI = proton pump inhibitor.

Eligibility Criteria:

HP-infected and uninfected male and female individuals of all age groups were included in this study. Individuals with infections other than *H. pylori*, pregnant women, Wilson's disease, thyroid disease, diabetes, pernicious anemia, and severely sick (intensive care unit-ICU, patients on hemodialysis, individuals with end-stage renal disease (ESRD) state, chronic kidney disease patients) patients were excluded. Severe HP-infected ICU patients stabilized by proton pump inhibitors (PPI) for the long term were also excluded. Individuals with abnormal calciotropic hormones: calcitonin, Fibroblast Growth Factor 23 (FGF23), biologically active 1,25-dihydroxyvitamin D [1,25$(OH)_2$D], and parathyroid hormone (PTH), were not included in this research.

Sample Size Assessment:

The sample size (n) was estimated by employing the mathematical equation $n = N/(1+Ne^2)$ considering the margin of error (ME) of 0.05 (Yamane 1967). The ideal sample size (n=400) was evaluated for the present study. However, a sum of n = 385 samples met the eligibility criteria streamlined for this research.

Case/Operational Identification and Definition:

Hypercalcemia (> 2.6 mmol/L), hypocalcemia (< 2.2 mmol/L) (Tinawi 2021), low alkaline phosphatase (<30 U/L), high alkaline phosphatase (> 125 U/L) (Kozic *et al.* 2011; Mutua *et al.* 2018), hypomagnesemia (< 0.75 mmol/l), hypermagnesemia (> 0.95 mmol/l) (Micke *et al.*, 2021), hypophosphatemia (< 0.81 mmol/L), hyperphosphatemia (> 1.45 mmol/L) (García Martín *et al.* 2020), hypoalbuminemia (< 35 g/l), and hyperalbuminemia (> 50 g/l) (Busher 1990).

Laboratory Technique and Generation of Data:

The collection of stool and blood specimens was carried out as per the standard operating procedure of the laboratory at the hospital to generate the primary data. Stool samples were processed to characterize the HP positive and negative individuals. The rapid immuno-diagnosis was undertaken by HP stool antigen (HPSA) test by employing an Immuno-Card STAT procedure. Following the separation of serum from blood specimen liver function tests (LFT), and bone profile: serum calcium, calcium, serum albumin, alkaline phosphatase, and serum magnesium were evaluated by using a DxC 700 AU analyzer (Beckman Coulter, USA) whereas determining complete blood count (CBC), a hematology system (ADVIA 2120i) was used. Serum ferritin was evaluated by a DxI 800 analyzer (Beckman Coulter, USA). Hypoalbuminemia usually results in hypocalcemia because of the reduction in protein-bound Ca^{+2}. Therefore, corrected calcium concerning albumin level was evaluated using the standard formula: corrected total serum Ca^{+2} (mmol/L) = evaluated serum Ca^{+2} in mg/dl + 0.02 (40 - serum albumin in g/L) (Parent *et al.*, 2009; Tinawi 2021).

Analytical Processing and Interpretation:

The data stratification was accomplished based on gender (males and females) and exposure type (HP-infected and HP-uninfected). HP-stool antigen-positive participants were considered as exposed and HP-stool antigen-negative individuals were identified as unexposed groups. Descriptive analytical processing of the data was undertaken using R-studio-v-4.0.2. The median (IQR) was illustrated in the form of boxplots. For the categorical variables frequency, proportions, and *p*-values were computed by executing Chi-square test, VCramer (for effect size estimation), Bayesian posterior VCramer (effect size confirmation) with 95% high-density interval (HDI), and bias factor/log (BF_{01}) using ggstatsbar. The tidyverse package of R-studio (v-4.3.1)/R-base was used for computing the inferential statistical processing: chi-square test, t-test, univariate, and multivariate logistic regressions (binary and multinomial). The cut-off value for significance was set to be < 0.05 for the level of statistical significance. For representation/plotting, ggplot2 and GGally packages were used.

RESULTS

The Fundamental Characteristics of All the Participants:

The mean ± SDs for corrected calcium, uncorrected calcium, magnesium, phosphorous, albumin, and ALP of overall participants were 2.2 ± 0.3, 2.2 ± 0.3, 0.8 ± 0.1, 1.2 ± 0.3, 39.2 ± 7.2, and 73.2 ± 31.8 respectively (Table 1). The mean ± SDs (female vs male) and *p*-values for corrected calcium, uncorrected calcium, magnesium, phosphorous, albumin, and ALP were (2.2 ± 0.3 vs 2.3 ± 0.3; $p = 0.077$), (2.2 ± 0.3 vs 2.3 ± 0.3; $p < 0.001$), (0.83 ± 0.1 vs 0.81 ± 0.1; $p = 0.4$), (1.31 ± 0.3 vs 1.21 ±

0.3; p = 0.9), (37.7 ± 7.8 vs 40.7 ± 6.2; p <0.001), and (69.3 ± 26.8 vs 77.1 ± 35.7; p = 0.016) respectively (Table 1). The mean ± SDs (*HP*-infected versus HP-uninfected) and p-values for corrected calcium, uncorrected calcium, magnesium, phosphorous, albumin, and ALP were (2.17 ± 0.3 vs 2.19 ± 0.3; p = 0.04), (2.2 ± 0.3 vs 2.2 ± 0.3; p = 0.5), (0.82 ± 0.1 vs 0.84 ± 0.1; p = 0.8), (1.23 ± 0.3 vs 1.22 ± 0.3; p = 0.2), (39.0 ± 7.2 vs 39.4 ± 7.2; p =0.6), and (73.5 ± 29.6 vs 77.1 ± 72.9 ± 33.8; p = 0.9) respectively (Table 1). The mean ± SDs for age, hemoglobin, MCV, MCHC, AST, ALT, RBC, Platelets, WBC, and MCHC of overall participants, by gender and by *H. pylori* infection status with level of statistical significance are summarized in Table 1. The comparative analyses of the bone profile components by *HP*-infection scenarios in terms of median, interquartile range (IQR) are illustrated in Figure 2a (corrected calcium), 2b (uncorrected calcium), 2c (magnesium), and in Figure 3a (albumin), 3b (ALP), and 3c (phosphorus).

Table 1. Summary of baseline characteristics of overall, male, female, HP-infected, and HP-uninfected participants (n = 385).

Characteristic	Overall status	Status by Gender			Status by HP infection		
	Overall (N = 385[1])	Female (N = 192[1])	Male (N = 193[1])	p-value[2]	HP-uninfected (N = 195[1])	HP-infected (N = 190[1])	p-value[2]
AGE	44.4 ± 14.0	44.6 ± 13.9	44.1 ± 14.2	0.7	43.4 ± 14.5	45.4 ± 13.4	0.2
HB (g/dl)	14.5 ± 1.9	13.2 ± 1.5	15.7 ± 1.4	<0.001	14.5 ± 1.7	14.5 ± 2.1	>0.9
MCV (Fl)	85.1 ± 6.9	84.0 ± 7.7	86.1 ± 6.0	0.003	85.0 ± 6.6	85.2 ± 7.3	0.8
MCH (Pg)	27.9 ± 11.2	27.8 ± 15.7	27.9 ± 2.4	>0.9	27.5 ± 2.9	28.3 ± 15.7	0.5
MCHC (g/dl)	32.1 ± 1.8	31.8 ± 2.1	32.4 ± 1.4	<0.001	32.3 ± 1.9	31.8 ± 1.6	0.014
PLT (10^9/L)	268.7 ± 75.0	293.5 ± 79.2	244.1 ± 61.6	<0.001	274.4 ± 73.1	262.9 ± 76.7	0.13
WBC (10^9/L)	6.0 ± 1.7	6.0 ± 1.7	5.9 ± 1.8	0.6	5.8 ± 1.6	6.1 ± 1.8	0.078
RBC (10^{12}/L)	5.3 ± 0.6	5.0 ± 0.5	5.6 ± 0.5	<0.001	5.3 ± 0.6	5.3 ± 0.6	0.4
ALT (U/L)	24.1 ± 17.0	17.3 ± 7.7	31.0 ± 20.6	<0.001	22.3 ± 18.0	26.0 ± 15.6	0.034
AST (U/L)	23.8 ± 8.6	21.1 ± 6.6	26.6 ± 9.4	<0.001	23.1 ± 8.0	24.6 ± 9.1	0.11
Alkaline phosphatase (U/L)	73.2 ± 31.8	69.3 ± 26.8	77.1 ± 35.7	0.016	72.9 ± 33.8	73.5 ± 29.6	0.9
Uncorrected calcium (mmol/L)	2.2 ± 0.3	2.2 ± 0.3	2.3 ± 0.3	<0.001	2.2 ± 0.3	2.2 ± 0.3	0.5
Albumin (g/L)	39.2 ± 7.2	37.7 ± 7.8	40.7 ± 6.2	<0.001	39.4 ± 7.2	39.0 ± 7.2	0.6
Magnesium (mmol/L)	0.8 ± 0.1	0.83 ± 0.1	0.81 ± 0.1	0.4	0.84 ± 0.1	0.82 ± 0.1	0.8
Phosphorus (mmol/L)	1.2 ± 0.3	1.21 ± 0.3	1.31 ± 0.3	0.9	1.22 ± 0.3	1.23 ± 0.3	0.2
Corrected calcium (mmol/L)	2.2 ± 0.3	2.2 ± 0.3	2.3 ± 0.3	0.077	2.19 ± 0.3	2.17 ± 0.3	0.4
[1]Mean ± SD;							
[2]Welch Two Sample t-test							

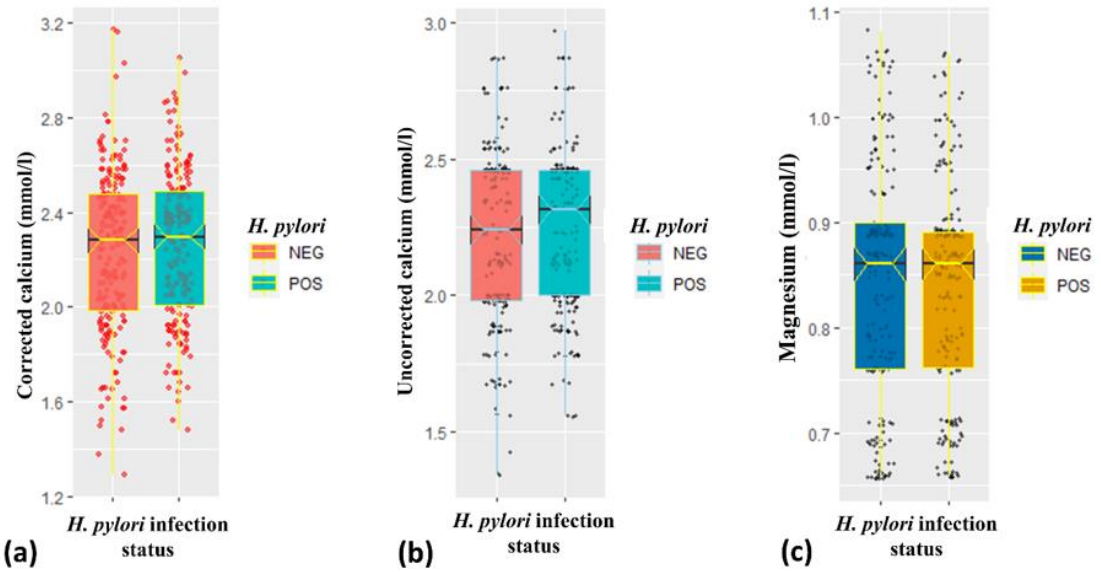

Fig. 2. Comparative illustration of components of bone profile by HP-infection status; (a)-status of corrected calcium, (b)-status of uncorrected calcium, and (c)-status of magnesium by HP-infection; POS = *H. pylori*-positive, NEG = *H. pylori*-negative.

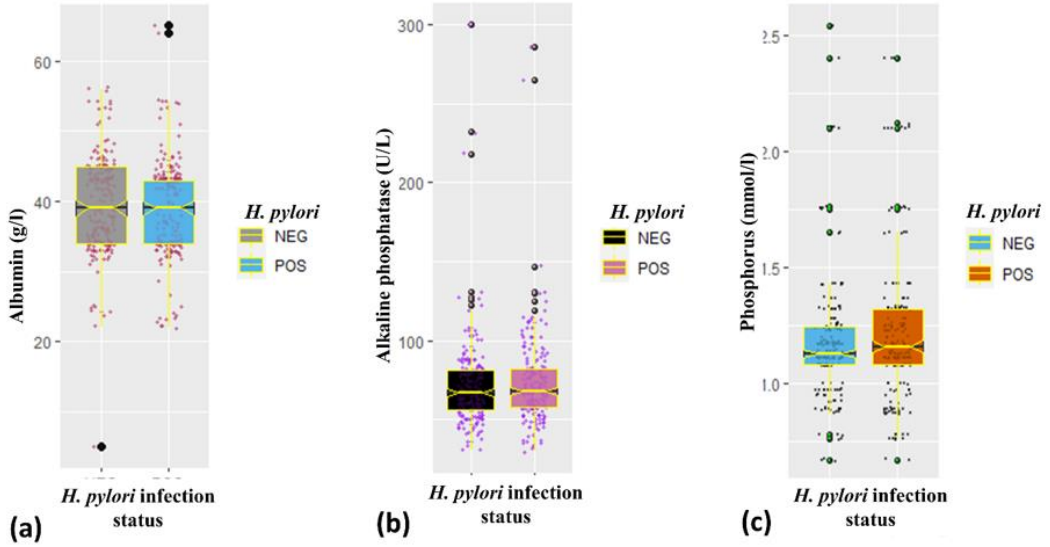

Fig. 3. Comparative illustration of components of bone profile by HP-infection status; (a)-status of albumin, (b)-status of alkaline phosphatase, and (c)-status of phosphorus by HP-infection; POS = *H. pylori*-positive, NEG = *H. pylori*-negative.

Proportional Distribution of HP-Associated Alterations in The Vital Components of The Bone Profile:

The status of the calcium: hypercalcemia, hypocalcemia, and normal calcium level (based on corrected and uncorrected calcium) were independent of the *HP*-infection as the p-value turned out to be > 0.05, effect size computed ranging from 0.00 to 0.1 (negligible association) in Vcramer and Bayesian V_{Cramer} tests, and the BF computed ranging from 3.4 to 4.6 as strong evidence of null hypothesis (based on corrected calcium: X^2 = 0.36; *p* = 0.83, V_{Cramer} = 0.00; 95%CI: 00, 1.00, Bayesian V_{Cramer} = 0.00; HDI: 0.0, 0.13, $BF_{(01)}$ = 3.75) (Fig. 4a) and (based on uncorrected calcium: X^2 = 0.08; *p* = 0.96, V_{Cramer} = 0.00; 95%CI: 00, 1.00, Bayesian V_{Cramer} = 0.00; HDI: 0.0, 0.12, $BF_{(01)}$ = 4.06) (Figure 4b). Based on corrected calcium, statistically insignificant differences were also measured in the proportion of hypercalcemia (N/%: 25/53% and N/%:22/47%, *p* = 0.66) and hypocalcemia (N/%: 82/48% and N/%:88/52%, *p* = 0.65) among HP-infected and HP-uninfected respectively (Figure 4a). The difference in the proportion of hypercalcemia and hypocalcemia among HP-infected vs uninfected based on uncorrected calcium is illustrated in Figure 4b. The magnesium status (hypermagnesemia and hypomagnesemia) and HP-infection status were independent of each other (X^2 = 0.32; *p* = 0.85, V_{Cramer} = 0.00; 95%CI: 00, 1.00, Bayesian V_{Cramer} = 0.04; HDI: 0.0, 0.13, $BF_{(01)}$ = 3.79) (Figure 5a) and so was the findings with albumin status (X^2 = 0.58; *p* = 0.75, V_{Cramer} = 0.00; 95%CI: 00, 1.00, Bayesian V_{Cramer} = 0.00; HDI: 0.0, 0.13, $BF_{(01)}$ = 3.97) (Figure 5b). The difference in the proportion of hypermagnesemia (N/%: 31/46% vs 36/54%; *p* > 0.05) and hypomagnesemia (N/%: 40/49% vs 41/51%; *p*> 0.05) is illustrated in Figure 5a, and that of hyperalbuminemia (N/%: 11/46% vs 13/54%; *p*> 0.05) and hypoalbuminemia(N/%: 61/52% vs 56/48%; *p*> 0.05) is depicted in Figure 5b among HP-infected and HP-uninfected participants. Hyperphosphatasemia and hypophosphatemia was not associated with HP infection significantly (X^2 = 1.65; *p* = 0.44, V_{Cramer} = 0.00; 95%CI: 00, 1.00, Bayesian V_{Cramer} = 0.04; HDI: 0.0, 0.16, $\log(BF_{01})$ = 3.83) (Fig. 6a) and increased ALP was weakly associated with HP infection (X^2 = 0.29; *p* = 0.59, V_{Cramer} = 0.00; 95%CI: 00, 1.00, Bayesian V_{Cramer} = 0.00; HDI: 0.0, 0.10, $BF_{(01)}$ = 2.97) (Fig. 6b). The variation in the proportions of hyperphosphatemia (N/%: 25/58% vs 18/42%; *p* > 0.05) and

hypophosphatemia (N/%: 12/52% vs 11/48%; $p > 0.05$) (Fig. 5a), and that in increased ALP concentration (N/%: 5/42% vs 7/58%; $p > 0.05$) (Fig. 5b) among HP-infected and HP-uninfected were observed.

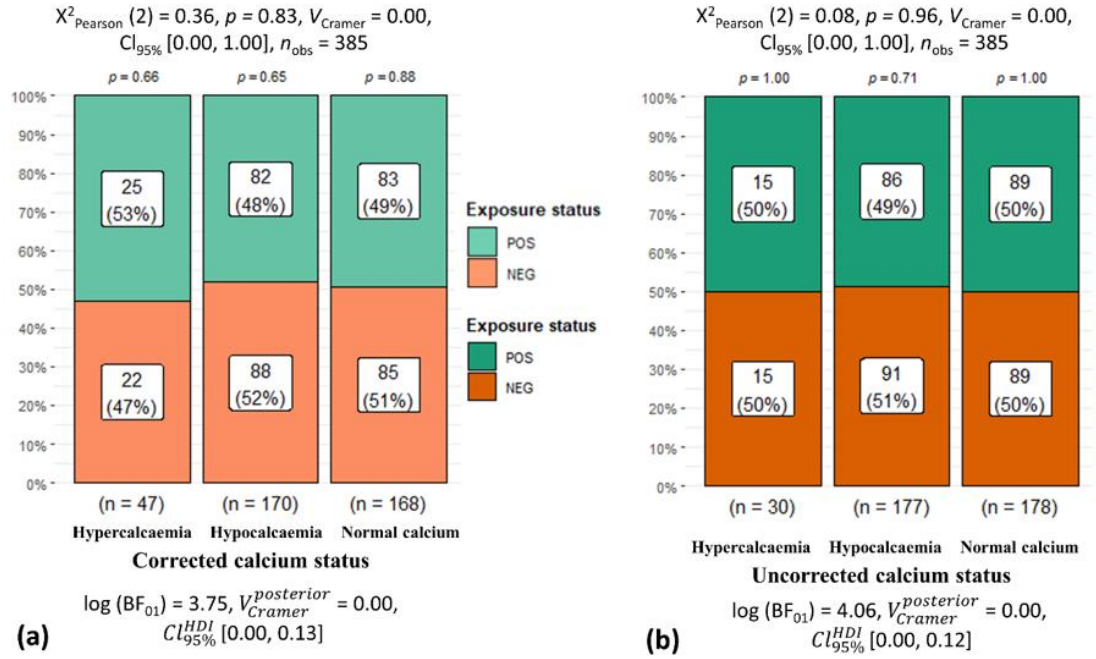

Fig. 4. A comprehensive illustration of the strength of association among the components of bone profile by HP-infection status; (a)-HP-associated alteration in calcium (based on corrected calcium), (b)-HP-associated alteration in calcium (based on uncorrected calcium); POS = *H. pylori*-positive, NEG = *H. pylori*-negative; n_{obs} = the number of observations, CI = confidence interval, BF = bias factor (0 = null and 1 = alternative hypotheses), p = probability, X^2 = chi-square, and HDI = high-density interval.

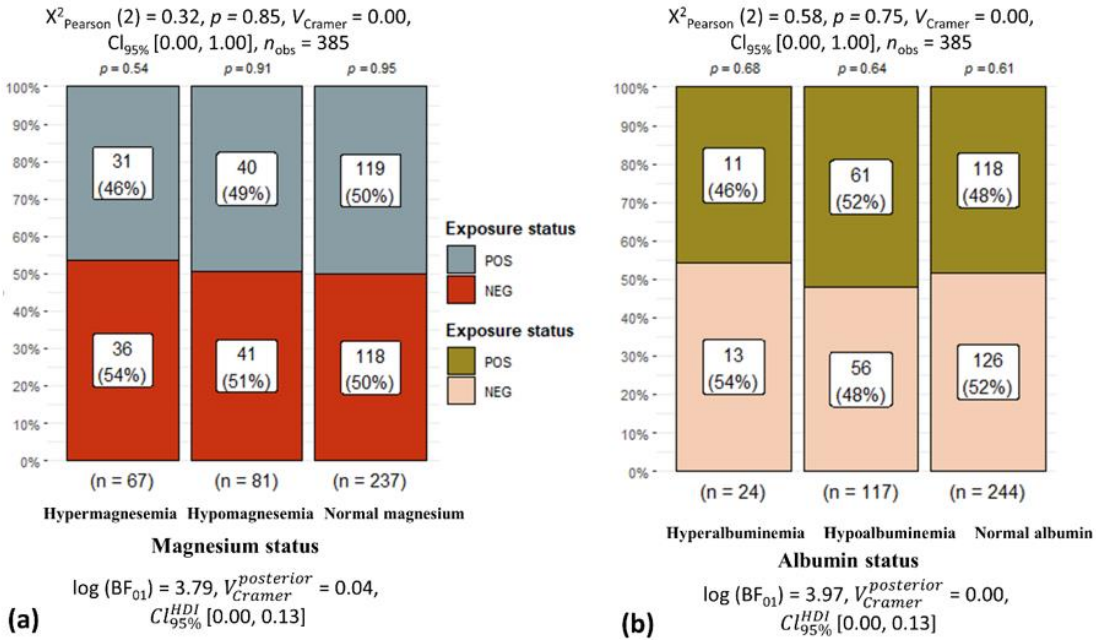

Fig. 5. A comprehensive representation of the strength of association among the components of bone profile by HP-infection status; (a)-HP-associated alteration in magnesium, (b)-HP-associated alteration in albumin; POS = *H. pylori*-positive, NEG = *H. pylori*-negative; n_{obs} = the number of observations, CI = confidence interval, BF = bias factor (0 = null and 1 = alternative hypotheses), p = probability, X^2 = chi-square, and HDI = high-density interval.

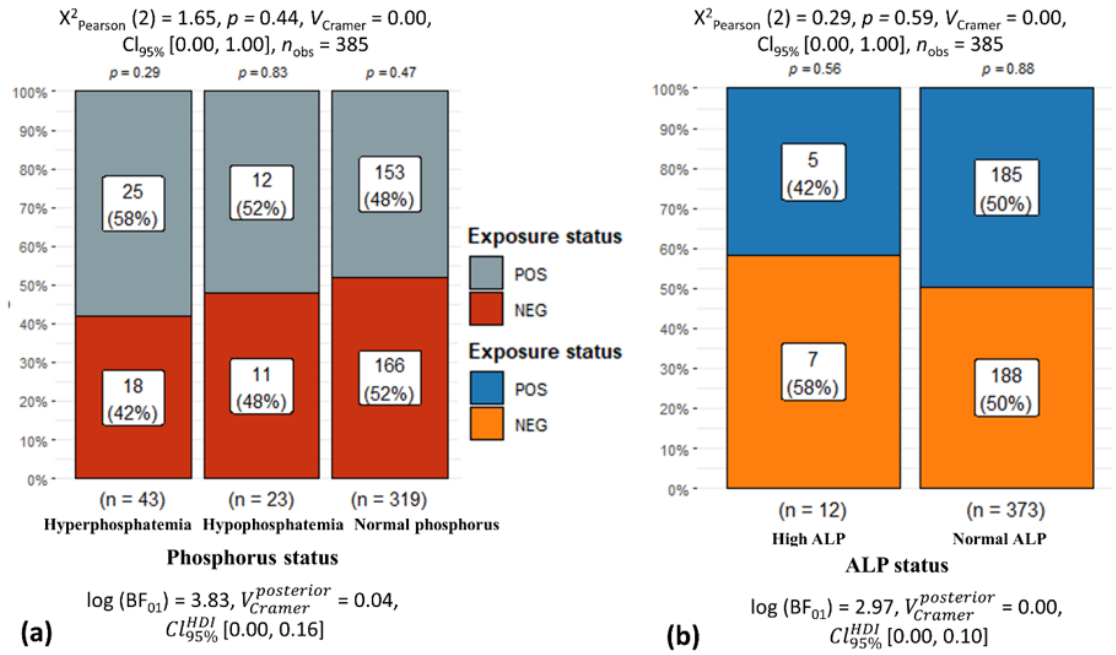

Fig. 6. A comprehensive illustration of the strength of association among the components of bone profile by HP-infection status; (a)-HP-associated alteration in phosphorus, (b)-HP-associated alteration in ALP; POS = *H. pylori*-positive, NEG = *H. pylori*-negative; ALP = alkaline phosphatase, n_{obs} = the number of observations, CI = confidence interval, BF = bias factor (0 = null and 1 = alternative hypotheses), p = probability, X^2 = chi-square, and HDI = high-density interval.

The Magnitude and Strength of *HP* Infection-Associated Alterations in Bone Profile Components of the HP-Infected Participants:

An overall correlation between corrected calcium and albumin was statistically significant (r = -0.351). Corrected calcium and albumin for HP-infected participants correlated with HP-uninfected groups was r = -0.373 and r = -0.30 respectively (Fig. 7). The magnesium correlated weakly with uncorrected calcium (r = 0.104) (Fig. 7). With one unit increase in corrected calcium, uncorrected calcium, and phosphorus odds of *HP*-infected as compared to an uninfected increase by 1.31 times (adjusted-OR: 1.31; CI (95%): 0.72–2.44; p > 0.05), 1.19 times (Adjusted-OR: 1.19; CI (95%): 0.60–2.37; p > 0.05), and 1.41 times (adjusted-OR:1.41; CI (95%): 0.72–2.79; p > 0.05) respectively (Table 2). Moreover, the odds of being *HP*-infected compared to uninfected individuals decrease by 25% (adjusted-OR: 0.75; CI (95%): 0.11–5.15; p > 0.05), and 1% (adjusted-OR: 0.99; CI (95%): 0.96–1.02; p > 0.05) with one unit increase in the concentration of magnesium and in that of albumin respectively as summarized in Table 2. The ALP concentration did not associate with *HP* infection significantly (adjusted OR: 1.0, CI(95%): 0.99–1.01; p > 0.05) (Table 2). The adjusted and unadjusted odds ratio with CI (95%) for other parameters such as Hb, MCV, MCHC, RBC, MCH, WBC, AST, age, and platelets are summarized in Table 2. In addition to that, the odds of being hyperphosphataemic against normal phosphorus level is 168% (adjusted OR: 2.68; CI (95%): 1.18–6.13; p < 0.05) greater in *HP*-infected compared to *HP*-uninfected (Table 3). Additionally, the odds of having hyperalbuminemia as compared to normal albumin level is 25% (adjusted OR: 1.25; CI (95%): 0.47–3.35; p < 0.001) more in the *HP*-infected compared to HP-uninfected group (Table 3). Furthermore, the odds of having hypercalcemia and hypocalcemia in comparison to normal calcium levels are

1.14 times (adjusted OR: 1.14; CI (95%): 0.53–2.14; $p > 0.05$) and 1.17 times (adjusted OR:1.17; CI (95%): 0.71–1.96; $p > 0.05$) higher in *HP*-infected compared to *HP*-uninfected individual respectively. The odds ratios for hypermagnesemia, hypomagnesemia, hypoalbuminemia, hypophosphatasemia, and high ALP were statistically insignificant (Table 3).

Fig. 7. A thorough illustration of the correlation matrix (Pearson's correlation) of the components of bone profile by HP-infection status; POS = *H. pylori*-positive, NEG = *H. pylori*-negative, ALP, Uca = uncorrected calcium, CCa = corrected calcium, Mg = magnesium, Corr = correlation coefficient (r), and P = phosphorus; the number of asterisks (*) denotes the level of statistical significance in that category; grey color denotes the overall correlation between variables while cyan and red colours denote correlation in HP infected and uninfected categories respectively.

Table 2. Tabulation of measures of association (logistic regression analyses) of *H. pylori* infection with the components of the bone profile and other laboratory parameters (n = 385)

Characteristic	Multivariate binary logistic regression			Univariate binary logistic regression		
	AOR[1]	95% CI[1]	p-value	COR[2]	95% CI[1]	p-value
MCV (Fl)	0.81	0.57, 0.86	0.2	1.00	0.98, 1.03	0.8
MCH (pg)	1.82	1.03, 5.34	0.2	1.01	0.99, 1.05	0.5
MCHC (g/dl)	0.44	0.17, 0.52	0.065	0.85	0.75, 0.97	0.017
Albumin modular (g/l)	0.99	0.96, 1.02	0.5	0.99	0.97, 1.02	0.6
HB (g/dl)	1.26	0.55, 3.03	0.6	1.00	0.90, 1.11	>0.9
PLT (10^9/L)	1.00	0.99, 1.00	0.035	1.00	1.00, 1.00	0.13
WBC (10^9/L)	1.16	1.02, 1.33	0.025	1.11	0.99, 1.25	0.080
RBC (10^{12}/L)	0.49	0.05, 4.57	0.5	1.15	0.83, 1.59	0.4
ALT (U/L)	1.01	0.99, 1.04	0.2	1.01	1.00, 1.03	0.041
AST (U/L)	1.00	0.96, 1.04	>0.9	1.02	1.00, 1.05	0.11
Alkaline phosphatase (U/L)	1.00	0.99, 1.01	0.7	1.00	0.99, 1.01	0.9
Uncorrected calcium (mmol/L)	1.19	0.60, 2.37	0.6	1.27	0.67, 2.43	0.5
Magnesium (mmol/L)	0.75	0.11, 5.15	0.8	0.76	0.12, 4.75	0.8
Phosphorus (mmol/L)	1.41	0.72, 2.79	0.3	1.45	0.78, 2.75	0.2
Corrected calcium (mmol/L)	1.31	0.72, 2.44	0.41	1.33	0.73, 2.45	0.4

[1]AOR = Adjusted Odds Ratio, [2]COR, = Crude Odds Ration, CI = Confidence Interval, reference category = *H. pylori* uninfected, AOR was obtained after adjustment for gender.

Table 3. Tabular representation of the strength of association (multinomial logistic regression analyses) of *H. pylori* infection with alteration in the components of the bone profile (*n* = 385).

Characteristic	Multivariate multinomial logistic regression			Univariate multinomial logistic regression		
	AOR[1]	95% CI[1]	p-value	COR[2]	95% CI[1]	p-value
Hypercalcemia (corrected Ca)	1.14	0.53, 2.14	0.82	1.08	0.53, 2.21	0.82
Hypocalcemia (corrected Ca)	1.17	0.71, 1.96	0..81	0.91	0.56, 1.47	0.7
Hypercalcemia (uncorrected Ca)	1.17	0.49, 2.80	0.72	1.09	0.47, 2.57	0.83
Hypocalcemia (uncorrected Ca)	1.23	0.76, 2.2	0.4	1.06	0.66, 1.69	0.081
Hypermagnesemia	0.88	0.47, 1.66	0.69	0.67	0.36, 1.25	0.2
Hypomagnesemia	1.13	0.63, 2.04	0.66	0.82	0.48, 1.42	0.5
Hyperphosphatemia	2.68	1.18, 6.13	0.01	2.78	1.24, 6.24	0.82
Hypophosphatemia	1.07	0.41, 2.84	0.88	1.08	0.24, 2.83	0.81
Hyperalbuminemia	1.25	0.47, 3.35	<0.001	0.59	0.22, 1.56	0.28
Hypoalbuminemia	1.35	0.79, 2.31	0.1	0.94	0.57, 1.56	0.82
High ALP	1.81	0.39, 8.5	0.4	0.52	0.13, 2.12	0.3

[1]AOR = Adjusted Odds Ratio, [2]COR, = Crude Odds Ration, CI = Confidence Interval, Ca = Calcium, ALP = Alkaline phosphatase, reference category in dependent variable = *H. pylori*-negative, reference categories in outcome variables: normal corrected calcium level, normal uncorrected calcium level normal phosphorus level, normal magnesium level, normal ALP level, and normal albumin level.

DISCUSSION

Substantial evidence is available to explain that the *HP* infection is associated with extra-gastric manifestations which is a multi-faceted health challenge (A G Gravina *et al.*, 2018). *HP* infection and their association with dermatological diseases (SU *et al.*, 2012), anemia (Al Mutawa *et al.*, 2023), dyslipidemia (Izhari *et al.*, 2023), allergic diseases (Arnold *et al.*, 2012), iron deficiency anemia (Al Mutawa *et al.* 2023), inflammatory bowel diseases (Song *et al.*, 2009), and bone disorder (Heidari 2015) has been investigated in recent years. Although only a few reports highlight the impact of *HP* infection on bone mineral density (BMD) (Pan *et al.*, 2018), and on osteoporosis (Wang *et al.*, 2019), to the best of my knowledge, *HP*-infection-associated alteration in the components of the bone profile of the HP infected individual is yet to be understood explicitly. In the current research, the association of alteration in bone profile components with *HP* infection was aimed to be investigated to gain insight into it. Statistically significant differences in albumin (mean ± SD: 37.7 ± 7.8 versus 40.7 ± 6.2: p <0.001), uncorrected calcium (mean ± SD: 2.2 ± 0.3 versus 2.3 ± 0.3: p <0.001), and ALP (mean ± SD: 69.3 ± 26.8 versus 77.1 ± 35.7: p = 0.016) levels in females vs males. Higher calcium level in males observed in this study was consistent with the results obtained by Liu et al. who reported also higher calcium concentration in males (mean ± SD: 2.32 ± 0.16 mmol/l) than in females (mean ± SD: 2.3 ± 0.159 mmol/l) with p-value < 0.001 (Liu *et al.*, 2022). Higher ALP concentration in males observed in this study corroborated the finding of Gordon *et al.* (Gordon 1993). However, the difference in the mean ± SDs of the components of the bone profile (HP-infected vs HP-uninfected) was statistically insignificant: corrected calcium (mean ± SD: 2.17 ± 0.4 vs 2.19 ± 0.4: p = 0.04), uncorrected calcium (mean ± SD: 2.2 ± 0.4 vs 2.2 ± 0.4: p = 0.5), magnesium (mean ± SD: 0.82 ± 0.1 vs 0.84 ± 0.1: p = 0.8), phosphorous (mean ± SD: 1.23 ± 0.3 vs 1.22 ± 0.31: p = 0.2), albumin (mean ± SD: 39.0 ± 7.2 vs 39.4 ± 7.2: p =0.6), and ALP (73.5 ± 29.6 vs 77.1 ± 72.9 ± 33.8: p = 0.9). Observations of this study corroborated with that of a study accomplished by Alirezaei *et al.*, (Alirezaei *et al.* 2017) who reported an insignificant differences in mean ± SDs of most of the components of the bone profile (magnesium: 2.979 ± 0.31 vs 3.011 ± 0.36, p = 0.797, calcium: 9.50 ± 0.440 vs 9.80 ± 1.8 p = 0.45, phosphorus: 5.091 ± 0.830 vs 4.760 ± 1.211 p = 0.324, and albumin: 4.21 ± 0.530 vs 4.11 ± 0.490 p = 0.65) between *HP*-infected and *HP*-uninfected groups

(Alirezaei *et al.* 2017). V_{Cramer}, Bayesian $V_{Cramer,}$ and Bias factor (BF) tests for the dependence of proportionality revealed that the proportion hypercalcemia, hypocalcemia, hypermagnesemia, hypomagnesemia, hyperphosphatemia, and hypophosphatemia in *HP*-infected and *HP*-uninfected categories were not significant (p >0.05). Moreover, insignificant difference in the proportion of hyperalbuminemia, hypoalbuminemia, and high-ALP was observed in *HP*-infected and *HP*-uninfected groups (p >0.05). Simoes et al. explicitly explained the scanty evidence of the *HP* infection-associated alteration in concentration of macronutrients and micronutrients (Simões *et al.*, 2022). The odds of being hyperphosphataemic as compared to normal phosphorus level is 168% (adjusted OR: 2.68, CI (95%): 1.18–6.13; $p < 0.05$) more in the *HP*-infected compared to the uninfected group (Table 3). Previous report described that the *HP* infection correlated with concentration of serum phosphorus significantly (Baradaran & Nasri 2005). Additionally, the odds of having hyperalbuminemia as compared to normal albumin level is 25% (adjusted OR:1.25; CI (95%): 0.47–3.35; $p < 0.001$) greater in *HP*-infected compared to uninfected-group (Table 3) which corroborated with the similar observations made in other studies (Jalalzadeh *et al.*, 2010). Hypercalcemia, hypocalcemia, hypermagnesemia, hypomagnesemia, hypoalbuminemia, and high-ALP did not associate with the *HP* infection which corroborated with observations of a study accomplished by Simoes et al. who advocated for the necessity of thorough investigation on *H. pylori*-associated alteration in calcium, phosphorus and Magnesium (Simões *et al.*, 2022).

Conclusion

Extra-gastrointestinal manifestations of *HP* infection are multifaceted health problems. To the best of my knowledge, the *HP* infection-associated alteration in components of the bone profile of the infected individuals has been investigated for the first time in this region. *HP* infections are associated significantly with phosphorus (hyperphosphatemia) and serum albumin (hyperalbuminemia). However, evidence of significant association of *HP* infection with alteration in calcium (hyper/hypocalcemia), magnesium (Hyper/hypomagnesemia), and ALP was not found. large group well-controlled studies are recommended on various components of bone profile to gain deep insight and to understand the HP infections and their impact on bone disorders such as altered bone mineral density and osteoporosis. The association of *HP* infections with hyperphosphatemia is suggestive of the modulation in bone mineral density which could affect the bone health of *HP*-infected patients, therefore, delineation of *HP*-associated modulation in the vital components of the bone profile and associated bone disorders in *HP*-infected patients could the future research directions, especially, in children.

Declarations:

Ethical Statement: The study received ethical approval from the Research Ethical Committee (REC) constituted at Armed Forces Hospital, in the Southern region, KSA. Approval details: reference code; AFHSRMREC, reference number; 2023/687, and approval date: 02/04/2023.

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