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STUDY OF SERUM HEPCIDIN LEVEL IN PATIENTS WITH COLORECTALCANCER AND ITS RELATIONSHIP TO TUMOR PROGRESSION

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ABSTRACT

Background: The incidence of colorectal cancer is rising worldwide. Several experimental studies have clarified the role of hepcidin as a new reliable marker that helps to predict colorectal cancer developmentand prognosis. Here we report our evaluation of hepcidin levels in patients diagnosed with colorectal cancer, study the significance of hepcidin levels as a marker for tumor progression and its correlation with the relapse in colorectal cancer. **Objectives**: Comparing hepcidin levels between healthy controls and colorectal cancer patients, studying the relationship between hepcidin levels and tumor stage in addition to evaluating its role as prognostic marker of colorectal cancer. **Materials and methods**: Our study included 39 patients admitted to Tishreen University Hospital in Lattakia and diagnosed with colorectal cancer. blood samples were collected before undergoing any type of treatment. Hepcidin levels in the study group were measured and compared to hepcidin values in the healthy control group comprising 12 patients. **Results:** Hepcidin levels were elevated in colorectal cancer patients compared to healthy controls (p-value< 0.05), with a significant difference between hepcidin levels in colorectal cancer patients according to the stage of the tumor (p- value< 0.05).

KEYWORDS: Hepcidin, Colorectal cancer, Relapse, Prognostic marker, Oncology.

1. INTRODUCTION

Colorectal cancer (CRC) is the third most popular occurring cancer in males after lung and prostate cancers and the second most commonly occurring cancer in females after breast cancer.[1] There were over 1.9 million new cases recorded in 2020, and it is considered the second most common cause of death from cancer, estimated to be responsible for approximately 935,000 cancer deaths per year.[1,2] There is a broad geographic variation in the incidence of CRC between different countries around the world, especially in countries that adopt the "western" lifestyle and diet.[3,4] It is estimated that by 2035, the mortality rate from colorectal cancer will increase by 60 to 71.5% (the estimate of these figures varies from country to country depending on the degree of economic development, access to treatment and environmental changes).[5]

Hepcidin (encoded by *Hamp* gene) is a 25-amino acid peptide hormone produced by the liver.[6] Although it is classified as an antimicrobial peptide, it has a crucial role in iron metabolism, as it is the main regulator of iron levels in the body.[7] The mechanism of action depends on the interaction of hepcidin with ferroportin (FPN), known to be the main iron export protein.[8] Hepcidin induces FPN degradation; therefore blocking iron export from cells such as enterocytes and macrophages.[9] High hepcidin levels also inhibit intestinal iron absorption by a similar mechanism.[10] Hepcidin secretion is controlled by several factors, including iron status, inflammation, anemia and erythropoietic process.[11-13]

Hepcidin levels increase in cancer patients under stimulation of inflammatory factors, in particular Interleukin-6, BMP6 and CRP, as well as other tumor-related regulatory factors. Elevated hepcidin acts by preventing iron absorption from the small intestine and blocking its release from macrophages, leading to disorders of iron metabolism, which are implicated in the iron deficiency anemia prevalent in cancer patients.[14,15] In colorectal cancer, several studies have reported elevated serum hepcidin compared to healthy individuals.[11] [16]

Indeed, high hepcidin levels results in increased intracellular iron sequestration, promoting tumor proliferation and migration by supporting the high metabolic demands during tumor progression.[17,18] Additionally, increased hepcidin levels also lead to the accumulation of iron in the colonocytes and this can

result in increased Wnt signaling which has been shown to be crucial in colorectal carcinogenesis.[19,20] Other potential mechanisms may also have been involved.[22,22] However, the role of hepcidin in cancer pathogenesis has not been confirmed, with some studies reporting no significant differences.[23,24] Our study seeks to explore the role of hepcidin in colorectal cancer (CRC) thoroughly, aiming to fill the gap in current knowledge. Specifically, we will compare hepcidin levels between healthy individuals and CRC patients, examine the association between hepcidin levels and disease stage, assess differences in hepcidin levels between patients who have experienced relapse and those who have not, and investigate the potential of hepcidin as a prognostic indicator for CRC.

2. MATERIALS AND METHODS

2.1 Sample Collection

Our study included 51 participants distributed as follows: 12 healthy controls and 39 patients diagnosed with colorectal cancer after admission to Tishreen University Hospital in Lattakia, Syria. Blood samples were drawn from patients upon diagnosis with colorectal cancer, before receiving any type of treatment, whether surgical, chemical or radiological. Hepcidin levels were measured using an ELISA kit from R&D Systems, Inc., a Bio-Techne brand.

The study took place from March 2022 to October 2022. Patients were then followed for 18 months to check for possible relapses or death.

Patients with chronic liver or kidney diseases, disorders of iron metabolism, sever nutritional deficiency, acute or chronic hemolytic lesions, inflammatory or infectious diseases were all excluded.

3. STATISTICAL ANALYSIS

- Statistical analyzes were conducted using the Statistical Package for the Social Sciences (SPSS), version 26. The Shapiro-Wilk test was performed to check thenormal distribution.
- Graphic forms and tables were used in the characterization of values. Averages, Standard Deviations and Central Tendency Measures were used to characterizequantitative data.
- The t-test was performed to see if the distribution was normal, and the Mann- Whitney U test for the non-normal distribution. The receiver operation characteristic curve (ROC Curve) was used to analyze the optimal cut-off value of hepcidin for predicting colorectal cancer. Results were considered statistically significant when

p-value < 0.05.

4. RESULTS

4.1 Sample Characteristic

After considering the previously mentioned criteria, the study sample included 51 participants divided into two groups as following; the first group included 39 patients diagnosed with colorectal cancer and consisted of 23 females 58.97% and 16 males 41.03%, the age of patients ranged from 48 to 78 years with a mean of 60.48±7.67 years. Control group included 12 women whom age ranged from 21 to 58 years with a mean of 31.3±10.9 years (Table1).

Colorectal cancer patients were chosen from all different stages (1,2,3) equally and all of them were followed for 18 months from the beginning of this study. 4 patients died during the follow-up. 8 patients relapsed 20.5%, of which 4 patients died, meanwhile 31 patients were non-relapsed 79.5% (Table1).

Table 1: Main Charachteristics of the study sample.

		CRC		Control
		N=39		N=12
Age (Mean ± SD)		60.48 ± 7.67		31.33 ± 10.9
Gender		N	%	
	Male	16	41.03	100% Females
	Female	23	58.97	
Relapse incidence		N	%	
	Yes	8	20.5	
	No	31	79.5	
Death incidence		N	%	
	Yes	4	10.25	
	No	35	89.75	

4.2 : Comparison of hepcidin levels between patients and the healthy controls

When performing t-test and comparing the hepcidin level between the CRC and control group we found a statistically significant difference in hepcidin levels between control and CRC patients (p-value $\leq$ 0.05). (Table 2)

Table 2: Hepcidin levels in colorectal cancer patients and healthy controls.

t-Test: Two-Sample Assuming Unequal Variances

	CRC Hepcidin (ng/ml)	*Control Hepcidin (ng/ml)*
Mean	44.7	5.56
Variance	1147.65	23.79
Observations	39	12
Hypothesized Mean Difference	0	
Df	43	
t Stat	6.98	
P(T<=t) one-tail	6.68E-09	
t Critical one-tail	1.68	
P(T<=t) two-tail	1.34E-08	
t Critical two-tail	2.01	

4.3 : Comparison of hepcidin levels between patients according to the stage ofcancer

There was a statistically significant difference between hepcidin levels among colorectal cancer patients according to the stage of tumor. We compared each two

stages separately (Tables 3,4) and noticed a strong correlation between colorectal cancer stage and hepcidin level.

Table 3: Hepcidin and colorectal cancer stages.
Anova: Single Factor

Hepcidin (*ng*/ml)				
Groups	***Count***	***Sum***	***Average***	***Variance***
Stage 1	13	144.7	11.13	11.92
Stage 2	13	463.8	35.67	88.15
Stage 3	13	1136.2	87.4	249.91

ANOVA

Source of Variation	***SS***	***Df***	***MS***	***F***	***p-value***	***F crit***
Between Groups	39410.74	2	19705.37	168.9	5.08E-19	3.25
Within Groups	4199.93	36	116.66			
Total	43610.67	38				

Table 4: Comparison between stages.

Comparison between stages		***p*- value**
Hepcidin ng/ml	1 vs 2	**0.0005**
	1 vs 3	**0.003**
	2 vs 3	**0.04**

4.4 : Comparison of hepcidin levels between replased and non-relapsed colorectal cancerpatients

Hepcidin levels were significantly higher in the relapsed group than non-relapsed group asshown in (Table 5) and (Fig 1).

Table 5: Hepcidin levels in relapsed and non-relapsed patients.

Hepcidin (*ng/ml*)		
	Relapsed	**Non-Relapsed**
Mean	76	36.6
Median	89.45	27.5
Std. Deviation	35.3	28.8
Minimum	15.4	5.6
Maximum	109.3	101.4
*p-value**	0.001	

***Shapiro-Wilk test**

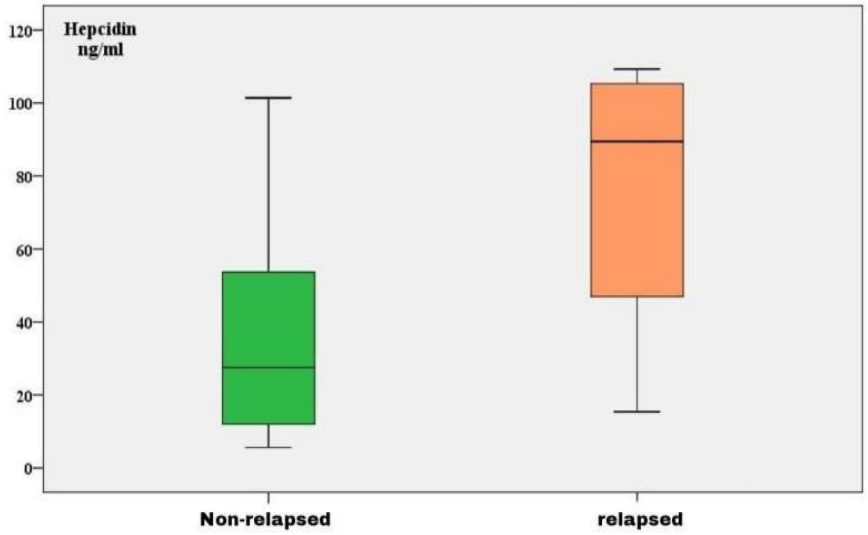

Figure 1: Hepcidin levels in relapsed and non-relapsed patients.

5. DISCUSSION

Colorectal cancer is one of the most common malignancies worldwide, significantly contributing to cancer morbidity and mortality.[25] The disease's progression and prognosis are influenced by various factors, including genetic mutations, lifestyle, and biomarkers.[26]

Hepcidin is a key regulator of iron homeostasis, primarily produced in the liver and influencing iron absorption and distribution in the body.[6] Elevated hepcidin levels have been associated with inflammation and malignancies, indicating its potential role as a biomarker incancer.[13]

Our study demonstrated a significant elevation in hepcidin levels in CRC group compared toControl group, with a *p-value* ≤0.05, corroborating previous international findings.

The notable increase in hepcidin levels observed in CRC group compared to Control group represents a significant discovery explicable through several underlying mechanisms associated with cancer pathophysiology and the regulation of iron metabolism:

Inflammatory Response: Cancer, including colorectal cancer, frequently triggers a persistent inflammatory condition marked by heightened production of cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-alpha).[27] These cytokines are known to stimulate hepcidin synthesis in the liver.[28] Hepcidin plays a crucial role in managing iron balance by inhibiting iron absorption in the intestine and iron release from macrophages, thereby decreasing iron availability to tumors and constraining their proliferation.[27,28]

Iron Sequestration and Anemia of Chronic Disease: Cancer-associated inflammation and the presence of tumors can induce a condition termed anemia of chronic disease (ACD) or anemia of inflammation.[29] In ACD, hepcidin levels rise as part of the body's response to inflammation, causing iron sequestration within macrophages and reduced iron absorption in the intestines.[30] This mechanism serves as a defensive response to curtail iron accessibility to pathogens and cancer cells.[14,29]

Tumor Interaction: Tumor cells themselves can generate factors that provoke hepcidin expression.[31] In this regard, tumor cells may discharge inflammatory mediators or other signaling molecules that directly or indirectly stimulate hepcidin production in hepatocytes.[32] This localized production of hepcidin contributes to the overall systemic increase observed[33] Additionally, tumor cells secrete hepcidin, which plays a significant role in iron sequestration.[34] By producing hepcidin, tumor cells trap iron within themselves, creating an iron-rich environment that supports their growth and development.[35] This mechanism not only facilitates the rapid proliferation of cancer cells but also aids in their survival and continued progression.[36]

Clinical Implications: Elevated hepcidin levels in colorectal cancer patients may bear clinical implications beyond iron metabolism.[37] Hepcidin has been scrutinized as a potential biomarker for detecting cancer[20], assessing its progression, and gauging responses to treatment.[38] Monitoring hepcidin levels alongside traditional tumor markers could furnish valuable insights into disease status and prognosis.[37]

In summary, the escalation of hepcidin levels in colorectal cancer patients mirrors a complex reaction involving cancer-induced inflammation, systemic mechanisms governing iron regulation, and potentially direct impacts of tumor-derived signals on hepatocytes.

Understanding these mechanisms is pivotal for devising targeted therapies and refining diagnostic approaches in the management of colorectal cancer.

This elevation in hepcidin has been documented in several studies, indicating its role in cancer-related iron metabolism. For instance, Nemeth *et al*. reported increased hepcidin levels in patients with various malignancies, linking it to cancer-induced anemia and inflammation.[11] Similarly, Ward et al. found elevated hepcidin levels in colorectal cancer patients, which supports our findings.[16]

The observed increase in hepcidin levels with tumor progression can be attributed to several factors. First, advanced tumors induce a more pronounced inflammatory response, leading to heightened production of pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-alpha), which stimulate hepcidin synthesis in the liver.[39] Second, the altered iron metabolism in advanced cancer stages results in upregulated hepcidin levels as the body attempts to sequester iron, limiting its availability to both the tumor and the cancer cells.[40] Additionally, the evolving tumor microenvironment, involving various cell types like immune cells, fibroblasts, and endothelial cells, contributes to increased hepcidin expression through the release of signaling molecules.[41] The relationship between increasing tumor burden and elevated hepcidin levels also suggests the body's effort to restrict iron availability, which is essential for tumor growth and proliferation.[42]

In our study, we found a statistically significant difference in hepcidin levels across differenttumor stages, the same findings were demonstrated by PAN XIANG-TAO (2017) and therby hepcidin could be a potential biomarker for assessing disease status and progression in colorectal cancer patients.[43]

Regarding the prognostic value of hepcidin, we observed a significant increase in hepcidin levels in patients who experienced relapse compared to those who did not, highlighting its potential as a prognostic marker. This is in line with the findings of Ward et al., who reported that elevated hepcidin levels could predict poor prognosis and relapse in colorectal cancer patients.[16] Additionally, other studies indicated the prognostic significance of hepcidin in cancer, associating higher levels with increased risk of relapse and reduced survival rates.[37,44,45]

In conclusion, our study confirms that hepcidin levels are significantly elevated in colorectal cancer patients compared to healthy individuals, with levels increasing in correlation with tumor stage and relapse. These findings underscore the importance of hepcidin as a biomarker for disease progression and prognosis, aligning with most existing literature while also pointing to the necessity for further investigation to resolve conflicting results.

6. CONCLUSION AND RECOMMENDATIONS

Through our study, the results were consistent with the role of hepcidin as a tumor marker incolorectal cancer, as well as a prognostic indicator during the course of the disease.

Future studies are recommended to evaluate the role of hepcidin among a larger number of patients to follow up hepcidin level in function of treatment regimens and response in order to determine hepcidin significance importance as a predictive marker of response to treatment.

Furthermore, measurement of other iron regulators in addition to hepcidin would help assess the risk and mechanism or iron deficiency associated with colorectal cancer.

7. LIMITATIONS

Our research is subject to certain limitations:

- The sample size was relatively small due to financial constraints and difficulties importing test kits into Syria, which may have impacted the significance of some findings. Additionally, the patient monitoring period was relatively brief, limited to the duration of the master's program. Comprehensive, long-term studies would yieldmore accurate results.
- We believe it is crucial to measure other regulators of hepcidin and iron, particularly Interleukin-6, to understand the mechanisms driving hepcidin upregulation in breast cancer. Regrettably, we were unable to pursue this objective due to the same limitations previously mentioned.

8. Ethical Approval

This research received approval from the scientific research ethics committee at Tishreen University and Tishreen University Hospital.

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10. REFERENCES

1. Siegel, R.L., et al., *Cancer statistics, 2023.* Ca Cancer J Clin., 2023; 73(1): 17-48.
2. Ahmed, M., *Colon cancer: a clinician's perspective in 2019.* Gastroenterologyresearch, 2020; 13(1): 1.
3. Watson, A.J. and P.D. Collins, *Colon cancer: a civilization disorder.* Digestive diseases, 2011; 29(2): 222-228.
4. Slattery, M.L., et al., *Lifestyle and colon cancer: an assessment of factors associatedwith risk.* American journal of epidemiology, 1999; 150(8): 869-877.
5. Favoriti, P., et al., *Worldwide burden of colorectal cancer: a review.* Updates insurgery, 2016; 68: 7-11.
6. Ganz, T., *Hepcidin and iron regulation, 10 years later.* Blood, The Journal of theAmerican Society of Hematology, 2011; 117(17): 4425-4433.
7. Ganz, T. and E. Nemeth, *Hepcidin and iron homeostasis.* Biochimica et Biophysica Acta (BBA)-Molecular Cell Research, 2012; 1823(9): 1434-1443.
8. Oates, P.S., *The role of hepcidin and ferroportin in iron absorption.* Histology andhistopathology, 2007.
9. De Domenico, I., et al., *The molecular mechanism of hepcidin-mediated ferroportin down-regulation.* Molecular biology of the cell, 2007; 18(7): 2569-2578.
10. Pagani, A., et al., *Hepcidin and anemia: a tight relationship.* Frontiers in physiology, 2019; 10: 494963.
11. Ganz, T., *Hepcidin, a key regulator of iron metabolism and mediator of anemia ofinflammation.* Blood, 2003; 102(3): 783-788.
12. Agarwal, A.K. and J. Yee, *Hepcidin.* Advances in chronic kidney disease, 2019; 26(4): 298-305.
13. Nemeth, E. and T. Ganz, *Hepcidin and iron-loading anemias.* Haematologica, 2006; 91(6): 727-732.
14. Madeddu, C., et al., *Pathogenesis and treatment options of cancer related anemia: perspective for a targeted mechanism-based approach.* Frontiers in physiology, 2018; 9: 1294.
15. Xu, Y., V.M. Alfaro-Magallanes, and J.L. Babitt, *Physiological and pathophysiologicalmechanisms of hepcidin regulation: clinical implications for iron disorders.* British Journal of Haematology, 2021; 193(5): 882-893.
16. Ward, D.G., et al., *Increased hepcidin expression in colorectal carcinogenesis.* World journal of gastroenterology: WJG, 2008; 14(9): 1339.
17. Pietrangelo, A., *Hepcidin in human iron disorders: therapeutic implications.* Journal of Hepatology, 2011; 54(1): 173-181.
18. Wu, X.-N., et al., *Roles of the hepcidin–ferroportin axis and iron in cancer.* European journal of cancer prevention, 2014; 23(2): 122-133.
19. Schwartz, A.J., et al., *Hepcidin sequesters iron to sustain nucleotide metabolism and mitochondrial function in colorectal cancer epithelial cells.* Nature metabolism, 2021; 3(7): 969-982.
20. Sornjai, W., et al., *Iron and hepcidin mediate human colorectal cancer cell growth.* Chemico-Biological Interactions, 2020; 319: 109021.
21. Henry, D.H., *Parenteral iron therapy in cancer-associated anemia.* Hematology 2010, the American Society of Hematology Education Program Book, 2010; 2010(1): 351-356.
22. Gilreath, J.A., D.D. Stenehjem, and G.M. Rodgers, *Diagnosis and treatment of cancer-related anemia.* American journal of hematology, 2014; 89(2): 203-212.
23. Wang, J., et al., *Hepcidin downregulation correlates with disease aggressiveness and immune infiltration in liver cancers.* Frontiers in oncology, 2021; 11: 714756.
24. Joachim, J.H. and K.J. Mehta, *Hepcidin in hepatocellular carcinoma.* British Journal ofCancer, 2022; 127(2): 185-192.
25. Siegel, R.L., et al., *Colorectal cancer statistics,*

2023. CA: a cancer journal for clinicians, 2023; 73(3): 233-254.
26. Bhasin, D. and A.L. Faulx, *What Are the Risk Factors for Colorectal Cancer?*, in *Curbside Consultation in GI Cancer for the Gastroenterologist*., CRC Press, 2024; 193-196.
27. Kartikasari, A.E., et al., *Tumor-induced inflammatory cytokines and the emerging diagnostic devices for cancer detection and prognosis.* Frontiers in oncology, 2021; 11: 692142.
28. Zhao, N., A.-S. Zhang, and C.A. Enns, *Iron regulation by hepcidin.* The Journal of clinical investigation, 2013; 123(6): 2337-2343.
29. Sears, D.A., *Anemia of chronic disease.* Medical Clinics of North America, 1992; 76(3): 567-579.
30. Eleftheriadis, T., et al. *The role of hepcidin in iron homeostasis and anemia in hemodialysis patients*. in *Seminars in dialysis*. 2009. Wiley Online Library.
31. Zhao, B., et al., *Role of hepcidin and iron metabolism in the onset of prostate cancer.* Oncology letters, 2018; 15(6): 9953-9958.
32. Fan, Y., et al., *Hepcidin upregulation in lung cancer: a potential therapeutic target associated with immune infiltration.* Frontiers in Immunology, 2021; 12: 612144.
33. Wang, D. and R.N. DuBois, *Immunosuppression associated with chronic inflammation in the tumor microenvironment.* Carcinogenesis, 2015; 36(10): 1085-1093.
34. Huang, L., et al., *Iron metabolism in colorectal cancer*. Frontiers in Oncology, 2023; 13: 1098501.
35. Ohta, K., et al., *Role of hepcidin upregulation and proteolytic cleavage of ferroportin 1 in hepatitis C virus-induced iron accumulation.* PLoS Pathogens, 2023; 19(8): e1011591.
36. Lelièvre, P., et al., *Iron dysregulation in human cancer: altered metabolism, biomarkers for diagnosis, prognosis, monitoring and rationale for therapy.* Cancers, 2020; 12(12): 3524.
37. Kali, A., M.V.P. Charles, and R.S.K. Seetharam, *Hepcidin-A novel biomarker with changing trends.* Pharmacognosy reviews, 2015; 9(17): 35.
38. Hare, D.J., *Hepcidin: a real-time biomarker of iron need*. Metallomics, 2017; 9(6): 606-618.
39. Strassmann, G., et al., *Evidence for the involvement of interleukin 6 in experimental cancer cachexia.* The Journal of clinical investigation, 1992; 89(5): 1681-1684.
40. Torti, S.V. and F.M. Torti, *Iron: The cancer connection.* Molecular aspects of medicine, 2020; 75: 100860.
41. Iyengar, N.M., et al., *Obesity and cancer mechanisms: tumor microenvironment and inflammation.* Journal of clinical oncology, 2016; 34(35): 4270.
42. Weiss, G., *Iron and immunity: a double-edged sword*. European journal of clinical investigation, 2002; 32: 70-78.
43. Xiang-Tao, P., *Expression of hepcidin and neogenin in colorectal cancer.* Open Medicine, 2017; 12(1): 184-188.
44. Chen, Q., et al., *Increased hepcidin expression in non-small cell lung cancer tissue and serum is associated with clinical stage.* Thoracic Cancer, 2014; 5(1): 14-24.
45. Phillips, E., et al., *A potential role for hepcidin in obesity-driven colorectal tumourigenesis.* Oncology reports, 2018; 39(1): 392-400.