High Seroprevalence of Hepatitis C Virus Antibody in Breast Cancer Patients in Egypt

Osama Hussein1, Engy Mohamed El-Ghitany2, Mawadda Omran3, Ghadier Matariek4, Esraa Ahmed Elbadaly3, Rana Hamdy5, Amira Gamal3, Mai Mohamed Zayed4, Ahmed Nasr3, Omar Hamdy1, Mohamed Elbasiony6 and Khaled Abdelwahab1

1Oncology Center and Department of Surgery, Faculty of Medicine, Mansoura University, Mansoura, Egypt. 2Tropical Health Department, High Institute of Public Health, Alexandria University, Alexandria, Egypt. 3Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt. 4Faculty of Pharmacy, Mansoura University, Mansoura, Egypt. 5Faculty of Science, Mansoura University, Mansoura, Egypt. 6Hepatology and Gastroenterology Unit, Internal Medicine Department, Mansoura University, Mansoura, Egypt.

ABSTRACT

BACKGROUND: Hepatitis C virus (HCV) is a known risk factor for hepatocellular carcinoma. Several epidemiological studies have pointed out to an association of HCV infection with other extrathepatic malignancies. The role of chronic HCV in breast cancer causation is less clear. Egypt is an endemic area of HCV infection with resulting significant morbidity. The association between HCV status and breast cancer risk in Egyptian women is hitherto unknown.

METHODS: A retrospective study was performed. The prevalence of anti-HCV seropositivity was estimated in a sample of women with a breast cancer diagnosis, retrieved from the hospital records, and was compared to the raw data of a population study in Egypt. Anti-HCV negative and positive patients were compared regarding the disease course and outcome.

RESULTS: Retrospective analysis revealed a markedly high prevalence of anti-HCV seropositivity in young breast cancer patients. In patients younger than 45 years, 13.4% were anti-HCV positive. Seropositivity was 6-fold higher in these patients than in adult females of the same age without cancer diagnosis (P=.003). The biological type, tumor size, nodal status, and disease-free survival were not affected by the patients’ HCV status.

CONCLUSION: Young Egyptian breast cancer patients have a dramatically high prevalence of HCV seropositivity. Further population studies are strongly required to investigate the epidemiological association of these two significant health problems.

KEYWORDS: Breast cancer, hepatitis C virus, Egypt, oncovirus

Background

Since the classic description of the “milk virus” in rodents,1 several authors have speculated a viral origin to breast cancer. Several groups have detected a similar sequence to the mouse mammary tumor virus (MMTV) in human breast cancer samples. Lawson and Glenn reviewed 17 publications that reported the examination of breast cancer tissues and normal breast samples for the presence of a conserved MMTV-specific sequence using polymerase chain reaction (PCR) technique.2 A significant correlation of the viral–specific sequence with tumor tissue was consistent across the studies. Based on their meta-analysis of these studies, Lawson and Glenn concluded that the MMTV-specific sequence had a 15-fold higher prevalence in breast cancer tissues relative to normal breast tissue control.

Other viruses have been isolated from the human breast tissue and a link to cancer causation was suggested. A recent meta-analysis of 30 case-control studies reported a pooled association of Epstein-Barr virus (EBV) with the risk of breast cancer. In that meta-analysis, The odds ratio was 4.74 (95% CI: 2.92–7.69; P<.0001).3 This and other meta-analyses confirmed an epidemiologic association of breast cancer with EBV.3,4 The human papillomavirus (HPV) is another DNA virus that has been evaluated for a possible breast cancer risk. Human papillomavirus is a well-known oncovirus with an established causal role in cancer of multiple mucosal sites.5 However, evidence of an association with breast cancer is limited to anecdotal reports.6,7 In recent years, the DNA of the bovine leukemia virus (BLV) was identified with increased frequency in breast cancer samples.8-10
In contrast to EBV and HPV, the BLV is a retrovirus that is oncogenic to cattle and has a great similarity to the human T-cell leukemia virus.11 In a retrospective study from MD Anderson Cancer Center, DNA of BLV was more likely to be found in breast cancer specimens compared to normal or benign breast tissue. More importantly, viral DNA was also more common in premalignant breast lesions.12 Although inconclusive, indications that breast cancer may be induced or at least facilitated by viral oncogenesis are still accumulating.

Hepatitis C virus is strongly associated with hepatocellular carcinoma (HCC), non-Hodgkin’s lymphoma (NHL), and to less extent, other cancers.13-15 The association of the virus with cancer incidence was particularly notable in geographical areas of endemic HCV infection.14,15 An association with breast cancer is elusive. A population-based, case-control study from Taiwan reported a prevalence rate of HCV infection affecting 2.9% and 2.4% of breast cancer patients and healthy controls, respectively. The authors found a significant 2-fold increase in breast cancer risk in HCV positive women younger than 50 years.16 In a longitudinal study from France, the authors reported a trend toward a higher prevalence of breast cancer in HCV-infected women that did not reach statistical significance.17 Several cohort studies failed to detect an association between breast cancer risk and HCV status.18-21 A US multicenter cohort of chronic liver patients was compared to Surveillance, Epidemiology, and End Results (SEER) data regarding cancer incidence, progression, and mortality. Hepatitis C virus-infected people had a 0.7 (95% CI = 0.6-0.8) standardized rate ratio of breast cancer compared to SEER’s (2006–2010) population. Age at diagnosis, stage, and cancer-related mortality did not statistically differ from the control group.18 Similarly, in a large Danish population cohort, the standardized incidence ratio of breast cancer in HCV patients was 0.25 (95% CI = 0.03–0.90).20

Given the uncertain relation of HCV infection with breast cancer risk, we undertook this study to determine the anti-HCV prevalence in breast cancer patients presented to a referral cancer hospital at Dakahlia, Egypt.

Methods
This is a retrospective study. The study protocol was prospectively registered on the US National Library of Medicine Clinical Trials registration system (ClinicalTrials.gov ID: NCT04090164) and was approved by the Faculty of Medicine Institutional Research Board (protocol #R.18.02.34.R1.R2). The hospital medical record system was searched to identify patients with the “malignant neoplasm of the breast” diagnosis. All consecutive patients with this diagnosis treated at the surgery department from January 2013 through December 2018 were identified. The list of identified patients was alphabetically arranged and the data of an unselected sample of those patients were retrieved from the system.

The sample size was calculated based on finite population size (the total number of breast cancer patients in the records), a hypothesized frequency of HCV 50% at 95% confidence level, and a design effect of 1, a sample of 343 breast cancer records needed for the study. To compensate for losses due to incomplete data, we included initially 650 records.

The following information was collected for all patients: name, age, hospital number, tumor stage information (T, N, M), tumor grade, tumor biological type, anti-HCV serological status at diagnosis, Hepatitis B surface antigen status at diagnosis, and the date and status at the last hospital visit.

Anti-HCV antibody was determined in patients’ sera using the third-generation enzyme-linked immunosorbent assay (ELISA; Biotech, UK). Mann-Whitney, chi-square, and log-rank tests were used for comparison of anti-HCV positive with anti-HCV negative patients regarding age, tumor characteristics, and disease-free survival (DFS), respectively.

The raw data from a published population-based study22 were used as a measurement of anti-HCV status in the non-cancer adult female population of Dakahlia (El-Ghitany; unpublished communication). Fisher’s exact test was used to compare the proportions of seropositive cases in the breast cancer cohort to that of the Dakahlia population.

A significant difference was considered when less than 5% of the difference might occur by chance (P < .05). Statistical calculations used Epi Info V.7 (www.cdc.gov), GraphPad Quick Calculator, and GraphPad Prism V.4 (GraphPad Software, Inc.) software.

Results
In total, 3125 breast cancer patients were identified in the hospital’s medical record. The information of an unselected sample of 650 patients was retrieved from the system; 245 patients with incomplete data were excluded, and the remaining 405 patients were subjected to further analysis.

In total, 88 patients (21.7%) were seropositive for HCV antibodies, 23 patients (5.7%) were hepatitis-B virus (HBV) positive, and none had concomitant HCV and HBV diagnosis. In patients younger than 45 years, anti-HCV seropositivity was detected in 17 out of 127 patients (13.4%). A significantly higher prevalence of anti-HCV (25.5%) was detected in patients 45 years or older (P < .01) (Table 1).

The pathological disease characteristics were similar in both groups (Table 2). At the time of analysis, median survival has not been reached. The DFS was similar in both anti-HCV positive and negative patients: hazard ratio = 1.650 (95% CI = 0.7388–3.207; P = .2494) (Figure 1).

Overall, 145 adult females from Dakahlia governorate were sampled in a published population-based cross-sectional study from 2015 to 2017.22 Anti-HCV positive women constituted 10.3% of cases. In women younger than 45 years, only 2.2% were seropositive for HCV.

The percentage of seropositive subjects was statistically higher in breast cancer patients than in adult female Dakahlia residents without a cancer diagnosis. The difference was observed in the total cohort of the adult female population and...
Table 1. Prevalence of anti-HCV serology in breast cancer patients.

|                      | HCV SERONEGATIVE | HCV SEROPosITIVE | P-VALUE |
|----------------------|------------------|------------------|---------|
| Median age (range)   | 49.0 (25-82)     | 55.5 (30-84)     | MW \(P < .0001\) |
| Patients < 45 years  | 110 (86.6%)      | 17 (13.4%)       | FE \(P = .0062\) |
| Patients > 45 years  | 207 (74.5%)      | 71 (25.5%)       |         |

Abbreviations: FE, Fisher’s exact test; HCV, hepatitis C virus; MW, Mann-Whitney test.

Hospital records were reviewed for the anti-HCV serology status of breast cancer patients at diagnosis. Positive anti-HCV serology correlated with older age at diagnosis.

Table 2. Clinicopathological criteria of breast cancers relative to anti-HCV serology status.

|                      | HCV SERONEGATIVE | HCV SEROPosITIVE | P-VALUE |
|----------------------|------------------|------------------|---------|
| Biological type      |                  |                  |         |
| HER2+                | 39 (12.3%)       | 14 (15.9%)       | \(\chi^2 P = .4814\) |
| Luminal A            | 144 (45.4%)      | 33 (37.5%)       |         |
| Luminal B            | 98 (30.9%)       | 31 (35.2%)       |         |
| TripleNegt           | 28 (8.8%)        | 10 (11.4%)       |         |
| Unknown              | 8 (2.5%)         | 0                |         |
| Clinical tumor size  |                  |                  |         |
| Tis                  | 2 (0.6%)         | 1 (1.1%)         | \(\chi^2 P = .8818\) |
| T1                   | 74 (23.3%)       | 25 (28.4%)       |         |
| T2                   | 206 (65.0%)      | 54 (61.4%)       |         |
| T3                   | 26 (8.2%)        | 7 (8.0%)         |         |
| T4                   | 5 (1.6%)         | 1 (1.1%)         |         |
| Unknown              | 4 (1.3%)         | 0                |         |
| Clinical node status |                  |                  |         |
| N0                   | 128 (40.4%)      | 27 (30.7%)       | \(\chi^2 P = .0446\) |
| N1                   | 174 (54.9%)      | 53 (60.2%)       |         |
| N2                   | 6 (1.9%)         | 6 (6.8%)         |         |
| N3                   | 4 (1.3%)         | 2 (2.3%)         |         |
| Unknown              | 5 (1.6%)         | 0                |         |
| Clinical node status |                  |                  |         |
| N0                   | 128 (40.4%)      | 27 (30.7%)       | FE \(P = .0840\) |
| N+                   | 184 (59.0%)      | 61 (69.3%)       |         |
| Grade                |                  |                  |         |
| GI                   | 4 (1.3%)         | 4 (4.5%)         | \(\chi^2 P = .0945\) |
| GIi                  | 228 (71.9%)      | 64 (72.7%)       |         |
| GIii                 | 71 (22.4%)       | 15 (17.0%)       |         |
| Unknown              | 14 (4.4%)        | 5 (5.7%)         |         |

Abbreviations: FE, Fisher’s exact test; HCV, hepatitis C virus; HER2, human epidermal growth factor receptor 2; TripleNegt, triple negative; \(\chi^2\), chi-square test.

Anti-HCV serology status did not affect the clinicopathological criteria of breast cancers.
was even more notable in younger patients ($P = .0027$ and $P = .003$, respectively) (Table 3).

**Discussion**

In this study, we observed a high prevalence of anti-HCV in breast cancer patients treated at a referral cancer hospital in the Egyptian governorate of Dakahlia. Seropositivity for anti-HCV was particularly high in younger patients, in striking difference from the healthy adult population in this locality. In breast cancer patients younger than 45 years, the percent of seropositive individuals was 6-fold higher than the reported percentage in adult female Dakahlia residents without a cancer diagnosis.

Egypt has recorded the highest global rate of (HCV) infection. Although the disease prevalence has been recently decreasing, persistent infection rates are still high and it is believed that morbidity related to the virus will continue through the next decades. Hepatitis C virus is an oncovirus and an established risk factor for HCC. The mechanism of HCV-induced liver carcinogenesis is less characterized than that of HBV. However, the high prevalence of HCV in the community linked most liver cancers in Egypt to HCV. Anti-HCV seropositivity affects 14.75% of Egyptian people. Males are much more affected than females. Chronic HCV is tightly linked to the age of the population and increases dramatically after the age of 44. In the 2015 Egyptian Health Issues Survey (EHIS), anti-HCV positive adult females constituted 9% and 14.8% of 40-44 year and 45-49 year female population, respectively. At the Dakahlia governorate, 2.2% of healthy adult females younger than 45 years tested positive for anti-HCV (El-Ghitany, unpublished communications), in sharp contrast with our data of women affected with breast cancer.

While data from countries with low infection rates failed to show an association of breast cancer with HCV, the association of the virus with cancer incidence was particularly notable in geographical areas of endemic HCV infection. A study from Taiwan linked HCV infection to breast cancer in younger patients. This population-based study found a non-significant increase in breast cancer risk in patients with viral hepatitis. However, HCV patients aged less than 50 years had a significant 2-fold higher risk of breast cancer in these authors’ experience. More recently, the same group conducted a population study to investigate the relation of HCV infection to colorectal cancer (CRC) risk. They detected a significantly higher prevalence of chronic HCV infection in CRC patients relative to matched controls. Similar to their earlier findings related to breast cancer, they reported a stronger association of CRC with HCV in patients younger than 45 years.

Currently, there is a well-defined association of HCV with HCC and NHL of B-cell origin (Table 4). Viral RNA is uncommonly detected in HCV-infected hepatocytes. While desirable, detection of viral RNA is not mandatory to indicate an oncogenic viral role. Hepatitis C virus can induce carcinogenesis through various mechanisms, including induction of reactive oxygen species, modulation of gene expression through viral micro RNA (miRNA), and manipulation of host immune response. Viruses maintain persistent infection

![Figure 1](image_url)
to the present study directly provides survival data for the first time in breast cancer patients with HCV seropositivity.

In this study, we did not confirm persistent HCV infection with PCR testing. However, the third-generation ELISA test is highly specific for the presence of HCV antibodies. In a cross-sectional study in a hospital setting, we cannot establish an epidemiologic causation between HCV and breast cancer. Further studies are needed to evaluate the impact of HCV infection on breast cancer risk in Egyptian women.

Conclusion
In this study, we confirmed a high prevalence of anti-HCV positivity in breast cancer patients, which was 6-fold higher than the prevalence in the healthy young adult female population. Our findings indicate the need for a case-control population study to determine the role of HCV as a risk for breast cancer.

Acknowledgements
The authors are indebted to the faculty and staff of Mansoura University Oncology Center for providing care to our patients.

Author Contributions
OH (first author) conceived the study, analyzed and interpreted the data, and wrote the manuscript. EME analyzed and interpreted the data and wrote the manuscript, and other authors collected the data. All authors discussed the data and approved the manuscript. MO, GM, EAE, RH, AG, MMZ, and AN contributed equally to this work.

Table 4. Oncogenic role of HCV.

| CANCER TYPE                     | REFERENCES |
|---------------------------------|------------|
| Hepatocellular carcinoma        | Mitchell et al,24 Goossens and Hoshida,25 |
| B-cell non-Hodgkin’s lymphoma   | Fiorino et al,19 Su et al,26 Zhu et al,27 Schöllkopf et al,28 and Carloni et al29 |

Abbreviation: HCV, hepatitis C virus.

through immune evasion strategies. The same strategies could prevent adequate tumor surveillance and induce proliferation. Induction of cytokine production is a major mechanism of HCV-induced lymphoproliferation and B-cell lymphoma formation.

Our findings indicate a significantly higher prevalence of anti-HCV positivity in breast cancer patients than in a healthy population and suggest the need for further investigations. It remains to be determined whether viral genomic material can be recovered from breast tissues and whether it is particularly abundant in cancer cells relative to normal breast cells. Although HCV infection was previously correlated with poor prognostic markers in Egyptian breast cancer patients, the present study directly provides survival data for the first time in breast cancer patients with HCV seropositivity.

Conclusion
In this study, we confirmed a high prevalence of anti-HCV seropositivity in Egyptian breast cancer patients, which was 6-fold higher than the prevalence in the healthy young adult female population. Our findings indicate the need for a case-control population study to determine the role of HCV as a risk for breast cancer.

Author Contributions
OH (first author) conceived the study, analyzed and interpreted the data, and wrote the manuscript. EME analyzed and interpreted the data and wrote the manuscript, and other authors collected the data. All authors discussed the data and approved the manuscript. MO, GM, EAE, RH, AG, MMZ, and AN contributed equally to this work.

Availability of Data and Material
The data at Ibn Sina Hospital Management System are not in the public domain. Data may be available from the corresponding author according to the Mansoura University policies.

Consent for Publication
Patients are not identified in the manuscript and their confidentiality has been all respected. Consent for publication is not applicable.

Ethical Approval and Consent to Participate
This is a retrospective study. The study was approved by the Mansoura Faculty of Medicine’s Institutional Research Board (protocol #R.18.02.34.R1.R2).

ORCID iD
Osama Hussein https://orcid.org/0000-0002-9563-0528

REFERENCES

1. Bittner JJ. Some possible effects of nursing on the mammary gland tumor incidence in mice. Science. 1936;84:162.
2. Lawson JS, Glenn WK. Evidence for a causal role by mouse mammary tumour-like virus in human breast cancer. NPJ Breast Cancer. 2019;5:40.
3. Farahmand M, Monavari SH, Shoja Z, Ghaffari H, Tavakoli M, Tavakoli A. Epstein-Barr virus and risk of breast cancer: a systematic review and meta-analysis. Future Oncol. 2019;15:2873-2885.
4. Huo Q, Zhang N, Yang Q. Epstein-Barr virus infection and sporadic breast cancer risk: a meta-analysis. PLoS ONE. 2012;7:e36556.
5. de Martel C, Plummer M, Vignat J, Franceschi S. Worldwide burden of cancer attributable to HPV by site, country and HPV type. Int J Cancer. 2017;141:664-670.
6. Mallone C, Longatto-Filho A, Filassi JR. Is human papilloma virus associated with breast cancer? A review of the molecular evidence. Acta Cytol. 2018;62:166-177.
7. Salman NA, Davies G, Majidy F, et al. Association of high risk human papillomavirus and breast cancer: a UK based study. Sci Rep. 2017;7:43591.
8. Martinez Cuesta L, Lendez PA, Nieto Farias MV, Dolcini GL, Ceriani MC. Can bovine leukemia virus be related to human breast cancer? A review of the evidence. J Mammary Gland Biol Neoplasia. 2018;23:101-107.
9. Buehring GC, Shen H, Schwartz DA, Lawson JS. Bovine leukemia virus linked to breast cancer in Australian women and identified before breast cancer development. PLoS ONE. 2017;12:e0179367.
10. Buehring GC, Shen HM, Jensen HM, Jin DL, Hudes M, Block G. Exposure to bovine leukemia virus is associated with breast cancer: a case-control study. PLoS ONE. 2015;10:e0134304.
11. Polat M, Takeshima SN, Aida Y. Epidemiology and genetic diversity of bovine leukemia virus. Front Cell. 2017;14:209.
12. Bolzelli KA, Shen HM, Krishnamurthy S, Sison JD, Nuovo GJ, Buehring GC. Bovine leukemia virus linked to breast cancer but not coinfection with human papillomavirus: case-control study of women in Texas. Cancer. 2018;124:1342-1349.
13. Fiorino S, Bacchi-Reggiani L, de Biese D, et al. Possible association between hepatitis C virus and malignancies different from hepatocellular carcinoma: a systematic review. World J Gastroenterol. 2015;21:12896-12953.
14. Liu B, Zhang Y, Li J, Zhang W. Hepatitis C virus and risk of extrahaematopoietic malignancies: a case-control study. Sci Rep. 2019;9:19444.
15. Su FH, Bai CH, Le TN, et al. Patients with chronic hepatitis C virus infection are at an increased risk of colorectal cancer: a nationwide population-based case-control study in Taiwan. Front Oncol. 2020;10:561420.
16. Su FH, Chang SN, Chen PC, Sung FC, Su CT, Yeh CC. Association between chronic viral hepatitis infection and breast cancer risk: a nationwide population-based case-control study. BMC Cancer. 2011;11:495.
17. Larrey D, Bozonnat MC, Kain I, Pageaux GP, Assenat E. Is chronic hepatitis C virus infection a risk factor for breast cancer? World J Gastroenterol. 2010;16:3687-3691.
18. Allison RD, Tong X, Moorman AC, et al. Increased incidence of cancer and cancer-related mortality among persons with chronic hepatitis C infection, 2006-2010. J Hepatol. 2015;63:822-828.
19. Amin J, Dore GJ, O’Connell DL, et al. Cancer incidence in people with hepatitis B or C infection: a large community-based linkage study. *J Hepatol*. 2006;45:197-203.

20. Omland LH, Farkas DK, Jepsen P, Obel N, Pedersen L. Hepatitis C virus infection and risk of cancer: a population-based cohort study. *Clin Epidemiol*. 2010;2:179-186.

21. Swart A, Burns L, Mao L, et al. The importance of blood-borne viruses in elevated cancer risk among opioid-dependent people: a population-based cohort study. *BMJ Open*. 2012;2:e001755.

22. El-Ghitany EM, Farghaly AG. Geospatial epidemiology of hepatitis C infection in Egypt 2017 by governorate. *Heliyon*. 2019;5:e02249.

23. El-Zanaty F. *Egypt Health Issues Survey*. Cairo, Egypt; Rockville, MD: Ministry of Health & Population, ICF International; 2015:1-234.

24. Mitchell JK, Lemon SM, McGivern DR. How do persistent infections with hepatitis C virus cause liver cancer? *Curr Opin Virol*. 2015;14:101-108.

25. Goossens N, Hoshida Y. Hepatitis C virus-induced hepatocellular carcinoma. *Clin Mol Hepatol*. 2015;21:105-114.

26. Su TH, Liu CJ, Tseng TC, et al. Hepatitis C viral infection increases the risk of lymphoid-neoplasms: a population-based cohort study. *Hepatology*. 2016;63:721-730.

27. Zhu X, Jing L, Li X. Hepatitis C virus infection is a risk factor for non-Hodgkin lymphoma: a MOOSE-compliant meta-analysis. *Medicine (Baltimore)*. 2019;98:e14755.

28. Schöllkopf C, Smelby KE, Hjalgrim H, et al. Hepatitis C infection and risk of malignant lymphoma. *Int J Cancer*. 2008;122:1885-1890.

29. Carloni G, Fioretti D, Rinaldi M, Ponzetto A. Heterogeneity and coexistence of oncogenic mechanisms involved in HCV-associated B-cell lymphomas. *Crit Rev Oncol Hematol*. 2019;138:156-171.

30. Machida K, Cheng KT, Sung VM, Lee KJ, Levine AM, Lai MM. Hepatitis C virus infection activates the immunologic (type II) isozyme of nitric oxide synthase and thereby enhances DNA damage and mutations of cellular genes. *J Virol*. 2004;78:8835-8843.

31. Gallo A, Miceli V, Bulati M, Iannolo G, Contino F, Conaldi PG. Viral miRNAs as active players and participants in tumorigenesis. *Cancers (Basel)*. 2020;12:358.

32. Krump NA, You J. Molecular mechanisms of viral oncogenesis in humans. *Nat Rev Microbiol*. 2018;16:684-698.

33. Attallah AM, El-Har M, Abddezak MA, et al. HCV nonstructural protein 4 is associated with aggressiveness features of breast cancer. *Breast Cancer*. 2018;25:297-302.

34. Colin C, Lanoir D, Touzet S, Meyaud-Kraemer L, Bailly F, Trepo C. Sensitivity and specificity of third-generation hepatitis C virus antibody detection assays: an analysis of the literature. *J Viral Hepat*. 2001;8:87-95.